Joint PhD degree in Environmental Technology

Docteur de l'Université Paris-Est
Spécialité : Science et Technique de l'Environnement

Dottore di ricerca in Tecnologie Ambientali

Degree of Doctor in Environmental Technology

Thesis for the degree of Doctor of Philosophy in Environmental Technology

Thèse – Tesi di Dottorato – PhD thesis – Väitöskirja

Andreina Laera

Fate of trace elements during and after anaerobic digestion: a sequential extraction method and DGT technique to assess bio-accessible trace elements in digestate

22/05/2019, Tampere

In front of the PhD evaluation committee

Prof. Gabriel Billon	Reviewer
Senior Scientist Dominique Patureau	Reviewer
Ass. Prof. Joyanto Routh	Reviewer
Prof. Eric D. van Hullebusch	Promotor
Prof. Giovanni Esposito	Co-promotor
Prof. Piet N.L. Lens	Co-promotor
Asst. Prof. Marika Kokko	Co-promotor
Prof. Gilles Guibaud	Chair,Co-promotor

Marie Sklodowska-Curie European Joint Doctorate, Advanced Biological Waste-to-Energy Technologies

Evaluation Committee

Chair

Prof. Gilles Guibaud
Equipe Développement d'indicateurs ou prévision de la qualité des eaux,
University of Limoges, PEIRENE
France

Reviewers/Examiners

Prof. Gabriel Billon
Laboratoire de Spectrochimie Infrarouge et Raman
Université de Lille
Villeneuve d'Ascq, France

Senior Scientist Dominique Patureau
Laboratoire de Biotechnologie de l'Environnement (LBE)
INRA, UR 0050,
Narbonne, France

Ass. Prof. Joyanto Routh
Department of Thematic Studies - Environmental Change
Linköping University
Linköping, Sweden

Prof. Gilles Guibaud
Equipe Développement d'indicateurs ou prévision de la qualité des eaux,
University of Limoges, PEIRENE
France

Thesis Promotor

Prof. Eric D. van Hullebusch
University of Paris-Est
France

Thesis Co-Promotors

Prof. Giovanni Esposito

Department of Civil and Mechanical Engineering
University of Cassino and Southern Lazio
Italy

Prof. Piet N.L. Lens
Department of Environmental Engineering and Water Technology
IHE Delft Institute for Water Education
The Netherlands

Asst. Prof. Marika Kokko
Faculty of Engineering and Natural Sciences
Tampere University
Finland

Prof. Gilles Guibaud
Equipe Développement d'indicateurs ou prévision de la qualité des eaux,
University of Limoges, PEIRENE
France

Supervisory team

Thesis Supervisor

Prof. Eric D. van Hullebusch
University of Paris-Est
France

Thesis Co-supervisors

Prof. Gilles Guibaud
Equipe Développement d'indicateurs ou prévision de la qualité des eaux,
University of Limoges, PEIRENE
France

Prof. Giovanni Esposito
Department of Civil and Mechanical Engineering
University of Cassino and Southern Lazio
Italy

Thesis Instructor

Ass. Prof. Rémy Buzier
Equipe Développement d'indicateurs ou prévision de la qualité des eaux,
University of Limoges, PEIRENE
France

This research was conducted in the framework of the Marie Sklodowska-Curie European Joint Doctorate (EJD) in Advanced Biological Waste-to-Energy Technologies (ABWET) and supported by from Horizon 2020 under grant agreement no. 643071.

Abstract

Different chemical interactions between trace elements and organic/inorganic compounds originating from the substrate and generated during the anaerobic digestion process will determine the speciation of trace elements in anaerobic digesters. After anaerobic digestion, digestates are exposed to oxidizing conditions which may favor a change of trace elements' speciation and consequently bio-accessibility for soil microorganisms and plants when digestates are spread on lands as organic amendment. Several techniques were used to assess the mobility, accessibility, and potential bio-availability of trace elements in digestates for environmental risk assessments of digestate utilization as a soil fertilizer. The aim of this thesis is to evaluate a sequential extraction procedure and the diffusive gradients in thin films technique (DGT) to assess bio-accessible trace elements in digestate samples. Samples were taken from full-scale anaerobic digestion plants treating a mixture of industrial and municipal solid wastes or sewage sludge. The elements investigated include Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Zn and W.

A sequential extraction procedure, originally conceived for organic matter fractionation, was implemented to simultaneously extract organic matter and trace elements in a substrate and digestate sample. It was observed that more than 60% of total As, Cd, Co, Fe, Mn, Ni and Zn were extracted along with the operationally defined organic matter fractions in both samples. In contrast, a lower recovery was observed for Al, Cr, Cu, Mo and Pb. These elements were mainly found in the dissolved organic matter fraction where soluble trace elements (*e.g.* free ions and complexed with organic/inorganic ligands) are likely bio-accessible for microbial up-take. Moreover, a high portion of elements was found in the mineral fraction (*e.g.* sulfide), which was considered poorly bio-accessible. However, the feasibility of using the aforementioned method was questioned following the low efficiency of extraction of certain trace elements during the extraction procedure. Moreover, it was acknowledged that chemical reagents employed during the extraction procedure could have promoted a dissolution/precipitation of trace elements and therefore a change in their fractionation.

Therefore, DGT technique was tested to fractionate trace elements and it was observed that this technique increased the sensitivity of trace elements monitoring compared to conventional dissolved elements measurements in digested sewage sludge. However, it was observed that the DGT samplers' deployment time in digested sewage sludge should be carefully evaluated. Additionally, the digestate matrix lowered the accumulation of some trace elements in the DGT samplers. Therefore, DGT labile trace elements

(*i.e.* most bio-accessible species) can be correctly estimated provided a careful adaptation of the deployment time as well as an evaluation of the matrix effect is performed in digestate samples. Unless this, general trend of labile trace elements over time could be estimated such as the distribution of labile trace elements over time in digestate exposed to air. Therefore, the effect of atmospheric air on the mobility and bio-accessibility of trace elements, including labile and soluble fractions, in digested sewage sludge was investigated. The exposure of digestate to air promoted dissolution of Al, As, Co, Cr, Cu, Fe, Mn, Mo and Pb, suggesting that a possible increase in their mobility may likely occur during digestate storage in open tanks or handling before land spreading. Labile elements' fraction increased only during an increase of aeration (except for Fe and Mn), suggesting that their short-term bio-accessibility can increase only after significant aeration as the one assumed to occur when digestate land spreading takes place.

These results open new fields of investigation for improving estimation of bio-accessible trace elements in digestate samples. For example, DGT technique should be further explored to accurately estimate labile trace elements concentrations in digestates.

Résumé

Différentes interactions chimiques entre les éléments traces métalliques (ETM) et les composés organiques/inorganiques provenant du substrat et générées au cours du processus de digestion anaérobie détermineront la spéciation des ETM dans les digesteurs anaérobies. Après digestion anaérobie, les digestats sont exposés à des conditions d'oxydation qui peuvent favoriser un changement de la spéciation des ETM et par conséquent de leur bioaccessibilité pour les microorganismes du sol et les plantes lors de l'épandage des digestats sur des terres agricoles en tant qu'amendement organique. Plusieurs techniques ont été utilisées pour évaluer la mobilité, l'accessibilité et la biodisponibilité potentielle des ETM dans les digestats afin d'évaluer les risques pour l'environnement liés à l'utilisation du digestat en tant qu'amendement organique. L'objectif de cette thèse est d'évaluer une procédure d'extraction séquentielle et la technique DGT pour évaluer les ETM bio-accessibles dans des échantillons de digestat. Les échantillons ont été prélevés dans des installations industrielles de digestion anaérobie, traitant un mélange de déchets solides industriels et municipaux ou de boues d'épuration. Les ETM étudiés sont : Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Zn et W.

Une procédure d'extraction séquentielle, conçue à l'origine pour le fractionnement de la matière organique (MO), a été mise en œuvre pour extraire simultanément la MO et les ETM dans un échantillon de substrat et de digestat. Il a été observé que plus de 60% des quantités totales d'As, Cd, Co, Fe, Mn, Ni et Zn étaient extraites avec les fractions de MO définies de manière opérationnelle dans les deux échantillons. En revanche, une extraction plus faible a été observée pour Al, Cr, Cu, Mo et Pb. Ces éléments étaient principalement présents dans la fraction de MO dissoute, où les ETM solubles (par exemple des ions libres et complexés avec des ligands organiques/inorganiques) sont probablement bio-accessibles pour l'absorption microbienne. De plus, une grande partie des éléments a été retrouvée dans la fraction minérale (par exemple, les sulfures), qui était considérée faiblement bio-accessible. Cependant, la possibilité d'utiliser la méthode susmentionnée a été remise en question par suite de la faible efficacité d'extraction de certains ETM au cours de la procédure d'extraction. De plus, il a été reconnu que les réactifs chimiques utilisés au cours de la procédure d'extraction auraient pu favoriser la dissolution/précipitation des ETM, donc une modification de leur fractionnement.

Par rapport aux mesures classiques de mesure des éléments dissous, la technique DGT augmente la sensibilité pour la mesure des ETM dans les boues d'épuration digérées. Cependant, il a été observé que le temps de déploiement des échantillonneurs DGT dans les boues d'épuration digérées devrait être soigneusement évalué. De plus, la matrice de digestat a réduit l'accumulation de certains ETM dans les échantillonneurs DGT.

Par conséquent, les ETM labiles de la DGT (c'est-à-dire la plupart des espèces bio-accessibles) peuvent être correctement estimés à condition d'adapter soigneusement le temps de déploiement et d'effectuer une évaluation des effets de matrice dans les digestats. L'évolution temporelle de la concentration des ETM labiles peut être estimée dans le digestat exposé à l'air. Par conséquent, l'effet de l'oxygénation des digestats sur la mobilité et la bio-accessibilité des ETM, y compris les fractions labiles et solubles, dans les boues d'épuration digérées a été étudié. L'exposition à l'air du digestat a favorisé la dissolution de Al, As, Co, Cr, Cu, Fe, Mn, Mo et Pb, suggérant une possible augmentation de leur mobilité qui pourrait probablement survenir lors du stockage du digestat dans des réservoirs ouverts ou lors de la manipulation avant l'épandage sur le sol. La fraction des éléments labiles n'augmente que pendant une aération prolongée (sauf pour Fe et Mn), ce qui suggère que leur bio-accessibilité à court terme ne peut augmenter qu'après une aération importante comme celle supposée se produire lors de l'épandage du digestat sur les sols.

Ces résultats ouvrent de nouveaux champs d'investigation pour améliorer l'estimation des ETM bio-accessibles dans les digestats. Par exemple, la technique DGT devrait être explorée pour estimer avec précision les concentrations en ETM labiles dans les digestats.

Tiivistelmä

Biokaasuprosessin syötteessä olevien tai siitä biokaasuprosessin aikana vapautuvien hivenaineiden sekä orgaanisten ja epäorgaanisten yhdisteiden kemialliset vuorovaikutukset vaikuttavat hivenaineiden jakautumiseen biokaasuprosessissa. Biokaasuprosessin jälkeen syntyvät mädätteet voivat altistua hapettaville olosuhteille, mikä voi edistää hivenaineiden jakautumista sekä niiden biosaatavuutta maaperän mikrobeille sekä kasveille, kun mädätettä levitetään pelloille orgaanisena maanparannusaineena. Tässä työssä mädätteiden hivenaineiden kulkeutumista ja biologista saatavuutta arvioitiin käyttämällä useita tekniikoita tavoitteena käyttää tätä tietoa ympäristöriskien arvioinnissa, kun mädätettä käytetään lannoitteena. Tämän opinnäytetyön tavoitteena on arvioida kahden eri teknologian, peräkkäisen uuttomenetelmän ja diffuusiogradientit ohuissa kalvoissa (DGT) –keräimen, käyttöä määrittämään biosaatavien hivenaineiden pitoisuuksia mädätteissä. Näytteitä otettiin täyden mittakaavan biokaasuprosesseista, joka käsittelivät sekä teollisuuden että yhteiskunnan jätteitä tai yhdyskuntajätevesilietettä. Tutkittuihin elementteihin kuuluivat Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Zn ja W.

Ensimmäisessä vaiheessa biokaasuprosessin substraatista sekä mädätteestä uutettiin samanaikaisesti orgaanista ainetta sekä hivenaineita peräkkäisellä uuttomenetelmällä, joka on alun perin kehitetty orgaanisen aineen erottamiseen eri jakeisiin. Yli 60% As, Cd, Co, Fe, Mn, Ni ja Zn –hivenaineista uutettiin molemmista näytteistä. Sitä vastoin Al, Cr, Cu, Mo ja Pb -hivenaineilla oli alhaisempi uuttotehokkuus. Näitä edellä mainittuja hivenaineita oli pääasiassa jakeessa, joka koostui liuenneesta orgaanisesta aineesta ja jossa liukoiset hivenaineet (esimerkiksi vapaat ionit ja ionit, jotka ovat ryhmittyneet orgaanisten tai inorgaanisten ligandien kanssa) ovat todennäköisesti biosaatavia mikrobeille. Lisäksi suuri osa hivenaineista oli mineraali-jakeessa (esim. sulfidit), jossa olevia hivenaineita pidettiin huonosti biosaatavana. Tämän menetelmän käyttö kyseenalaistettiin, koska tiettyjen hivenaineiden uuttotehokkuus oli pieni uuttoprosessin aikana. Lisäksi huomioitiin, että uuttoprosessin aikana käytetty kemikaalit ovat voineet lisätä hivenaineiden liukenemista tai saostumista, mikä on muuttanut niiden jakautumista eri jakeiden välillä.

Tästä syystä DGT-tekniikkaa tutkittiin hivenaineiden erottamiseen eri jakeisiin. Tämä tekniikka oli tarkkuudeltaan huomattavasti herkempi hivenaineiden pitoisuuksien määrittämiseen verrattuna perinteiseen liuenneiden hivenaineiden analysointiin mädätetystä jätevesilietteestä. DGT-näytteenottimen käyttöaika tulisi kuitenkin valita tarkasti. Lisäksi huomattiin, että mädäte vähensi joidenkin hivenaineiden kertymistä DGT-näytteenottimeen. DGT-menetelmällä voidaan kuitenkin arvioida labiilien

hivenaineiden (eli biosaatavimpien hivenaineiden) pitoisuuksia, jos käyttöaika valitaan oikein ja mädätteen aiheuttamat muutokset DGT-näytteenottimessa määritetään huolellisesti. DGT-menetelmällä pystyttiin arvioimaan labiilien hivenaineiden pitoisuuksia ajan suhteen mädätteestä, joka oli kosketuksissa ilman kanssa. DGT-menetelmällä määritettiinkin ilman vaikutukset labiilien ja liukoisten hivenaineiden pitoisuuksiin mädätetyssä jätevesilietteessä. Mädätteen altistaminen ilmalle lisäsi Al, As, Co, Cr, Cu, Fe, Mn, Mo ja Pb –hivenaineiden liukoisuutta, mikä viittaa siihen, että näiden hivenaineiden liikkuvuus voi kasvaa, jos mädätettä varastoidaan avoimissa altaissa tai jos mädäte pääsee kosketuksiin ilman kanssa ennen maaperään levittämistä. Vain ilmastuksen aikana havaittiin labiilien hivenaineiden pitoisuuksien kasvua (paitsi Fe ja Mn), mikä viittaa siihen, että useimpien hivenaineiden biosaatavuus kasvaa vain merkittävän ilmastuksen myötä (kuten mädätteen levityksen aikana).

Opinnäytetyön tulokset mahdollistavat uusien teknologioiden käytön, jotta voidaan paremmin määrittää biosaatavien hivenaineiden pitoisuuksia mädätteissä. DGT-tekniikkaa tulisi tutkia jatkossa lisää, jotta pystyttäisiin luotettavasti määrittämään labiilien hivenaineiden pitoisuuksia mädätteissä.

Sommario

Differenti interazioni chimiche tra gli elementi in traccia (ET) e composti organici/inorganici provenienti dal substrato e generati durante il processo di digestione anaerobica determineranno la speciazione degli ET nei digestori anaerobici. Dopo digestione anaerobica, i digestati sono esposti a condizioni di ossidazione che possono favorire un cambiamento della speciazione degli ET e conseguentemente della bio-accessibilità per i microrganismi del suolo e le piante quando il digestato è utilizzati come emendamento organico per il suolo. Diverse tecniche sono state utilizzate per valutare la mobilità, l'accessibilità e la potenziale bio-disponibilità di ET nei digestati per la valutazione del rischio ambientale di utilizzo del digestato come fertilizzante per il suolo. Lo scopo di questa tesi è di valutare una procedura di estrazione sequenziale e la tecnica DGT per valutare la bio-accessibilità di ET in campioni di digestato. Tali campioni sono stati prelevati da impianti industriali di digestione anaerobica che trattano una miscela di rifiuti solidi industriali e urbani o di fanghi di depurazione. Gli elementi studiati includono Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Zn e W.

Una procedura di estrazione sequenziale, originariamente sviluppata per il frazionamento della materia organica, è stata implementata per estrarre contemporaneamente materia organica e ET in un campione di substrato e digestato. È stato osservato che oltre il 60% del totale di As, Cd, Co, Fe, Mn, Ni e Zn sono estratti insieme alle frazioni di materia organica in entrambi i campioni. Al contrario, il recupero di Al, Cr, Cu, Mo e Pb è stato inferiore. Questi elementi sono stati estratti principalmente nella frazione di sostanza organica disciolta in cui gli ET disciolti (ad esempio ioni liberi e complessati con leganti organici/inorganici) sono probabilmente bio-accessibili per l’assorbimento microbico. Inoltre, una porzione elevata di elementi è stata trovata nella frazione minerale (ad esempio solfuro), che è stata considerata limitatamente bio-accessibile. Tuttavia, la fattibilità dell'uso del suddetto metodo è stata messa in discussione a seguito della scarsa efficienza dell'estrazione di alcuni ET durante la procedura di estrazione. Inoltre, è stato riconosciuto che i reagenti chimici impiegati durante la procedura di estrazione potrebbero aver promosso una dissoluzione/precipitazione di ET e quindi un cambiamento nel loro frazionamento.

Pertanto, la tecnica DGT è stata utilizzata per frazionare gli ET ed è stato osservato che questa tecnica ha aumentato la sensibilità del monitoraggio degli ET rispetto alle misure convenzionali di elementi disciolti dopo estrazione acida. Tuttavia, è stato osservato che il tempo di esposizione dei campionatori DGT nel digestato deve essere attentamente valutato. Inoltre, la matrice del digestato ha ridotto l'accumulazione di alcuni ET nei cam-

pionatori DGT. Pertanto, gli ET labili misurati dalla tecnica DGT (cioè la specie bio-disponibili) possono essere correttamente stimati a condizione che un accurato tempo di esposizione, nonché una valutazione dell'effetto matrice, venga stimato in campioni di digestato. Tuttavia, la tendenza generale degli ET labili nel tempo, come la distribuzione di ET labili nel tempo in digestato esposto a condizione di ossidazione, posso essere valutati. Pertanto, è stato studiato l'effetto dell'aria sulla mobilità e la bio-accessibilità degli ET, includendo le frazioni labili e solubili, nei fanghi di depurazione digeriti. L'esposizione del digestato all'aria ha promosso la dissoluzione di Al, As, Co, Cr, Cu, Fe, Mn, Mo e Pb, suggerendo che un possibile aumento della loro mobilità potrebbe verificarsi durante lo stoccaggio del digestato in vasche aperte o il trasporto e gestione del digestato prima della sua applicazione sul terreno. La frazione di elementi labili è aumentata solo durante un aumento dell'aerazione (eccetto Fe e Mn), suggerendo che la loro bio-accessibilità può aumentare solo dopo un'aerazione significativa come quella che si presume avvenga quando il digestato è applicato sul terreno.

Questi risultati aprono nuovi campi di indagine per migliorare la stima di ET bio-accessibili nel digestato. Ad esempio, la tecnica DGT dovrebbe essere ulteriormente perfezionata per stimare accuratamente le concentrazioni di ET labili nei digestati.

Samenvatting

Verschillende chemische interacties tussen sporenelementen en organische/anorganische verbindingen afkomstig van het substraat en gegenereerd tijdens het anaërobe vergistingsproces bepalen de speciatie van sporenelementen in anaërobe gistingstanks. Na anaërobe vergisting worden digestaten blootgesteld aan oxiderende omstandigheden die een verandering van de speciatie van sporenelementen, en bijgevolg ook de biologische toegankelijkheid, voor bodemmicro-organismen en planten kunnen bevorderen wanneer digestaten op het land worden verspreid. Verschillende technieken werden gebruikt om de mobiliteit, toegankelijkheid en potentiële biobeschikbaarheid van sporenelementen in digestaten te beoordelen voor milieurisicos van het gebruik van delfstoffen als bodemmeststof. Het doel van dit proefschrift is om een sequentiële extractieprocedure en de diffuse gradiënten in de dunne-filmtechniek (DGT) te evalueren om de biologisch toegankelijke sporenelementen in digestaatmonsters te beoordelen. Monsters werden genomen van volledige anaërobe vergistingsinstallaties die een mengsel van industriëel en gemeentelijk vast afval of rioolslib behandelden. De onderzochte elementen omvatten Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Zn en W.

Een sequentiële extractieprocedure, oorspronkelijk uitgewerkt voor fractionering van organische stoffen, werd geïmplementeerd om tegelijkertijd organisch materiaal en sporenelementen in een substraat en digestaatmonster te extraheren. Er werd waargenomen dat meer dan 60% van het totaal As, Cd, Co, Fe, Mn, Ni en Zn samen met de operationeel gedefinieerde organische stoffracties in beide monsters werden geëxtraheerd. Terwijl een lagere recovery werd waargenomen voor Al, Cr, Cu, Mo en Pb. Deze elementen werden voornamelijk aangetroffen in de fractie opgelost organisch materiaal waar oplosbare sporenelementen (bijvoorbeeld vrije ionen en gecomplexeerd met organische / anorganische liganden) waarschijnlijk bio-toegankelijk zijn voor microbiële opname. Bovendien werd een groot deel van de elementen gevonden in de minerale fractie (bijvoorbeeld sulfide), die als slecht biologisch toegankelijk werd beschouwd. De haalbaarheid van het gebruik van de bovengenoemde methode werd echter betwijfeld vanwege het lage rendement van de extractie van bepaalde sporenelementen tijdens de extractieprocedure. Bovendien werd erkend dat chemische reagentia die tijdens de extractieprocedure werden gebruikt een dissolutie/precipitatie van sporenelementen en daarmee een verandering in hun fractionering konden hebben bevorderd.

Daarom werd de DGT-techniek getest om sporenelementen te fractioneren en werd waargenomen dat deze techniek de gevoeligheid van sporenelementenbewaking verhoogde in vergelijking met conventionele opgeloste elementenmetingen in vergist

rioolslib. Er werd echter opgemerkt dat de opnametijd van de DGT-monsternemers in vergist rioolslib zorgvuldig moet worden geëvalueerd. Bovendien verlaagde de digestaatmatrix de accumulatie van enkele sporenelementen in de DGT-samplers. Daarom kunnen DGT labiele sporenelementen (dat wil zeggen, de meeste biologisch toegankelijke) correct worden geschat, mits een zorgvuldige aanpassing van de looptijd en een evaluatie van het matrixeffect wordt uitgevoerd in digestaatmonsters. Tenzij dit de algemene trend van labiele spoorelementen in de loop van de tijd zou kunnen schatten, zoals de verdeling van labiele sporenelementen in de tijd dat digestaat blootgesteld wordt aan de lucht. Daarom werd het effect van atmosferische lucht op de mobiliteit en biologische toegankelijkheid van sporenelementen, inclusief labiele en oplosbare fracties, in vergist rioolslib onderzocht. De blootstelling van digestaat aan lucht bevorderde de oplossing van Al, As, Co, Cr, Cu, Fe, Mn, Mo en Pb, wat suggereert dat een mogelijke toename van hun mobiliteit kan optreden tijdens de opslag van digestaat in open tanks of bij het verwerken voordat het op het land wordt verspreid. De fractie van de labiele elementen nam alleen toe tijdens een toename van de beluchting (behalve voor Fe en Mn), wat suggereert dat hun biobeschikbaarheid op korte termijn alleen kan toenemen na significante beluchting zoals bij de verspreiding van digestaat op het land.

De resultaten van dit proefschrift openen nieuwe onderzoeksrichtingen voor het verbeteren van de schatting van de biologisch toegankelijke sporenelementen in digestaatmonsters. De DGT-techniek moet bijvoorbeeld verder worden onderzocht naar hoe labiele sporenelementenconcentraties in digestaten nauwkeuriger kunnen worden geschat.

Acknowledgements

I would like to thank Prof. Eric D. van Hullebusch, Prof. Giovanni Esposito and the selection committee of the ABWET program for trusting my skills and for giving me the opportunity to grow as a researcher during these years. Moreover, I am grateful to Eric for letting me explore new scientific environments during conferences and meetings and, beyond my research project, the Marie Curie Alumni Association. I acknowledge the valuable support of Prof. Gilles Guibaud and Rémy Buzier to my research work and the memorable time I spent in Limoges. Rémy, your time and attention to my research work were valuable to improve my knowledge in the chemistry of trace elements and DGT technique. Gilles, I really appreciated your availability to discuss and review my research plans and to inspire me with new ideas. Overall, I am happy I challenged myself by speaking French with you.

I wish to thank Lena Lundman, Mårten Dario and Patrice Fondanèche for the precious assistance during the laboratory work. I thank all my ABWET colleagues for the nice time we spent together. I am very glad that I met Suchanya, Bikash, Anastasiia and Gilbert whit whom I spent most of my time and enjoyed the food and the landscapes of France and Italy. Moreover, the international mobility allowed me to meet many new friends in Paris, Limoges, Cassino and Linköping. I also would like to thank Prof. Bo Svensson and in particular Sepehr Shakeri Yekta for their encouraging support to begin my career as PhD researcher. I am really glad I met them and that we could collaborate during the doctorate.

I thank Maria Luisa Giordano for introducing me to meditation and for teaching me yoga that became part of daily life and helped me to find a balance during some critical moments. I thank Angelo Natale who gave me precious advices during the last few months of thesis writing.

A special thank goes to my parents, Giovanna and Anastasio, who always encouraged me to make steps forward and taught me to work with accuracy and dedication. They also encouraged me to travel and make new experiences away from the places where I grew up. I would like to thank my lovely friend Marina who has been always there for me. I also thank my friend Hana who always had positive and encouraging words for me. I also wish to thank ViChi for participating in most of my doctorate achievements, for supporting me when I was up and down and for strongly believing in me.

I finally would like to thank the European Union’s Horizon 2020 research and innovation programme for financing this project.

Italy, March 2019

Andreina Laera

To my perseverance, tenacity and spirit of adaptability

Contents

List of Symbols and Abbreviations

C_{DGT}	concentration of labile trace elements
C_e	concentration of trace elements in eluents
CH_4	methane
CPMAS	cross polarization magic angle spinning
CSH	carbonate, sulfides and hydroxides
D	diffusion coefficient
DGT	diffusive gradient in thin film technique
DMSO-d_6	deuterated dimethyl sulfoxide
DOM	dissolved organic matter
E_h	redox potential
EPS	extracellular polymeric substance
f_e	elution factor
HSQC	heteronuclear single quantum coherence
ICP-MS	inductively coupled plasma - mass spectrometry
MLD	method limit of detection
MLQ	method limit of quantification
MP-AES	microwave plasma - atomic emission spectrometer
N_2	nitrogen
NEOM	non-extractable organic matter
NMR	nuclear magnetic resonance spectroscopy
PEOM	poorly extractable organic matter

PES	polyethersulfone
PP	polypropylene
PPCO	polypropylene copolymer
REOM	readily extractable organic matter
SEOM	slowly extractable organic matter
SO_4^{2-}	sulfate
SPOM	extractable soluble from particulate organic matter
TS	total solids
TSS	total suspended solids
V_e	volume of the eluents
VFA	volatile fatty acids
VS	volatile solids
VSS	volatile suspended solids
Δ_{MDL}	thickness of the material diffusion layer

List of Publications

I. Laera, A., Shakeri Yekta, S., Hedenström, M., Buzier, R., Guibaud, G., Dario, M., Esposito, G., van Hullebusch, E.D., 2019. A simultaneous assessment of organic matter and trace elements bio-accessibility in substrate and digestate from an anaerobic digestion plant. *Submitted for publication*

II. Laera, A., Buzier, R., Guibaud, G., Esposito, G., van Hullebusch, E.D., 2019. Assessment of the DGT technique in digestate to fraction twelve trace elements. *Talanta* 192, 204–211.

III. Laera, A., Buzier, R., Guibaud, G., Esposito, G., van Hullebusch, E.D., 2019. Distribution trend of trace elements in digestate exposed to air: Laboratory-scale investigations using DGT-based fractionation. *Journal of Environmental Management* 238, 159–165.

Author's Contribution

I. Andreina Laera performed the experimental work and related analyses with the help of Mårten Dario for trace elements analysis and Mattias Hedenström for NMR analysis. She also analyzed data, prepared the manuscript and she is the corresponding author. Sepehr Shakeri Yekta participated in planning the experiment, helped with interpretation of data, with the writing and revised the manuscript. Mattias Hedenström also helped with NMR data interpretation and revised the manuscript. Rémy Buzier and Gilles Guibaud helped with interpretation of data and revised the manuscript. Giovanni Esposito and Eric D. van Hullebusch contributed in funding and planning the research, supervision and revision of the manuscript.

II. Andreina Laera performed the experimental work and related analyses with the help of Patrice Fondanèche for trace elements analysis. She also analyzed data and prepared the manuscript. Rémy Buzier is the corresponding author and participated in planning the experiment, helped with interpretation of data and with the writing and thoroughly revised the manuscript. Gilles Guibaud participated in planning the experiment, helped with interpretation of data and revised the manuscript. Giovanni Esposito and Eric D. van Hullebusch contributed in funding and planning the research, supervision and revision of the manuscript.

III. Andreina Laera performed the experimental work and related analyses with the help of Patrice Fondanèche for trace elements analysis. She also analyzed data and prepared the manuscript. Rémy Buzier is the corresponding author and participated in planning the experiment, helped with interpretation of data and with the writing and thoroughly revised the manuscript. Gilles Guibaud participated in planning the experiment, helped with interpretation of data and revised the manuscript. Giovanni Esposito and Eric D. van Hullebusch contributed in funding and planning the research, supervision and revision of the manuscript.

1 Introduction

Nowadays, the anaerobic digestion process is considered one of the best available techniques (European Commission, 2018a) for disposal of organic wastes and recovery of valuable by-products ergo biogas rich in methane (CH_4) and digestate. Moreover, anaerobic digestion is regarded as a recycling process capable of reducing the volume of organic wastes (European Commission, 2018b) which otherwise would be mostly destined to landfill or incineration.

Biogas and bio-methane are widely used for energy production (*i.e.* heat and electricity) and fuel for vehicles (Scarlat et al., 2018), whereas digestate is spread on agricultural land as amendment or fertilizer (Nkoa, 2014). Beside biogas, there are several advantages of using digestate as soil amendment, such as sequestering the carbon into soil and reducing carbon dioxide (CO_2) emissions in the atmosphere (Guintoli et al., 2017), improving the soil microflora and providing the majority of nutrients and organic matter to soils to enhance productivity (Tampio et al., 2016).

The agronomic value of digestate is well reported in the literature (Tambone et al., 2010, 2009; Tampio et al., 2016). It is observed that digestate slowly release nutrients to the soil compared to mineral fertilizers (Odlare et al., 2011). Moreover, the digestate organic matter is more stable than the raw organic material (Moeller, 2015) and therefore less unpleasant odors and gases are released to the atmosphere. However, despite the agronomic usefulness of digestate related to its organic matter content, the high concentration of trace metals such as cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) may preclude utilization of digestate for soil amendment (Bonetta et al., 2014; Kupper et al., 2014; Owamah et al., 2014; Tampio et al., 2016). For this reason, European countries, such as France and Italy (Ministero delle politiche agricole alimentari e Forestali, 2015; Ministre de l'agriculture et de l'alimentation, 2017), have adopted safety regulations to

ensure the quality of digestate before it is spread on land. However, to the best of our knowledge, a harmonized legislative framework for the use of digestate as soil amendment as well as common threshold values for trace element' concentrations do not exist yet. The revision of the European fertilizer regulation is currently ongoing[1]. Nevertheless, some threshold values for total Cd, Cr, Cu, Hg, Ni, Pb and Zn concentrations were set by the European commission for the use of sewage sludge in agriculture (European Commission, 1986).

Total elements estimation is a poor criterion to identify bio-accessible trace elements in digestate (van Hullebusch et al., 2016). In this context, bio-accessible fraction refers to compounds which can be used by microorganisms or roots in plants because they are not occluded or constrained in mineral particles (*i.e.* soluble compounds) (Semple et al., 2004). Only knowledge of speciation can help to assess bio-accessible trace elements in digestate and therefore the harm or benefit associated with digestate before spreading on agricultural land (van Hullebusch et al., 2016). Organic matter plays an important role in determining the chemical speciation of trace elements in anaerobic digester samples (Fermoso et al., 2015; Thanh et al., 2016). As an example, organic functional groups such as thiol groups (Shakeri Yekta et al., 2014b) can strongly complex trace elements making them less bio-accessible for microorganisms in anaerobic digestion systems. However, it is not well known which organic macromolecules in the anaerobic digester samples affect trace element bio-accessibility.

In recent years, the mobility and bio-accessibility of trace elements in digestate was investigated using sequential extractions procedures like the modified Tessier method (Ortner et al., 2014) or the Community Bureau of Reference (BCR) method (Cestonaro do Amaral et al., 2014). Alternatively, the diffusive gradients in thin films technique (DGT) was used to screen the presence of labile elements (*i.e.* the most readily bio-accessible forms of trace elements (Zhang and Davison, 2015)) in digested sewage sludge filtrate as reported by Takashima et al. (2018) for the first time.

In this thesis, a modified organic matter sequential extraction procedure (Jimenez et al., 2017, 2014) is applied to simultaneously extract organic matter and trace elements for assessment of the bio-accessibility of a combined source of carbon, energy and micronutrient trace elements in a substrate and digestate sample. Moreover, possible association of trace elements with the operationally defined organic matter fractions is investigated. After application of the sequential extraction method, DGT technique is used to

[1] https://eur-lex.europa.eu/procedure/EN/2016_84

assess labile trace elements in digested sewage sludge. The DGT technique is tested and adapted using digested sewage sludge and later it is used to assess the trend of labile trace elements over time in digestate exposed to air.

In the following background chapter, an overview of the chemical speciation of trace elements and the possible interactions with inorganic and organic ligands during the anaerobic digestion process are described. A summary of the current methods used to assess bio-accessible trace elements in anaerobic digester samples is also provided. The objectives and research questions of the thesis are specified in Chapter Three. An overview of the experiments, the methods and analytical approaches implemented to achieve the objectives of this research work are provided in Chapter Four. The outcomes are also presented in Chapter Four which also highlights the benefits and limitations of each fractionation method (*i.e.* sequential extraction procedure and DGT technique) to assess bio-accessible fractions of trace elements and organic matter. Moreover, recommendations are offered in Chapter Four to help establishing robust fractionation methods for the implementation of directives in the field of organic fertilizers for agricultural soils. The conclusions and future outlook are described in Chapter Five.

For a comprehensive description of material and methods along with the results, the reader can refer to the three papers attached at the end of this thesis. In particular, the sequential extraction method implemented to simultaneously assess organic matter and trace element bio-accessibility is presented in Paper I. Trace elements fractionation by DGT technique in digested sewage sludge is evaluated in Paper II. DGT-based fractionation to assess the distribution trend of trace elements in digestate exposed to air is implemented in Paper III.

2 Background

2.1 Trace elements chemistry in anaerobic digestors

Trace elements such as Co, Ni, Fe, Se and W are important nutrients for the growth and metabolism of bacteria and archaea during the anaerobic digestion process (Feng et al., 2010; Glass and Orphan, 2012; Takashima and Speece, 1989). Studies performed at laboratory scale (Feng et al., 2010; Gustavsson et al., 2013, 2011; Karlsson et al., 2012) demonstrated an improvement of CH_4 production and inhibition of volatile fatty acids (VFAs) production after addition of a single or a combination of trace elements (*i.e.* Co, Fe, Ni, Se, W) into anaerobic bioreactors by treating different types of substrate at hydraulic retention time (HRT) ranging from 20 to 30 days. Moreover, Lindorfer et al. (2012) reported an increase of biogas production in 60 anaerobic digestion plants located in Germany after addition of trace elements. Similarly, Vintiloiu et al. (2012) suggested a continuous supply of trace elements to improve the performance of full-scale anaerobic digestion plants.

The availability of trace elements for microbial uptake in digestate can be compromised mainly by the presence of sulfide (S^{2-}) and to a less extent by phosphate (PO_4^{3-}) and carbonate (CO_3^{2-}) which enable the precipitation of dissolved elements (Callander and Barford, 1983). Sulfide is mainly present as hydrogen sulfide (H_2S) species in the gas phase of anaerobic digesters (Callander and Barford, 1983) and Fe (III) ions are supplied to reduce H_2S levels in the biogas. Shakeri Yekta et al. (2012) observed that S was mainly precipitated as FeS according to the Sulfur K-edge X-ray absorption near-edge spectroscopy (XANES) analysis in the solid phase of a sludge sample from a laboratory scale anaerobic reactor. However, a small fraction of Fe was associated to reduced organic sulfur such as organic sulfide and thiol groups in the solid phase (Yekta et al.,

2012). Furthermore a study by Shakeri Yekta et al. (2014a), implemented with a thermodynamic equilibrium model, identified the chemical form (*i.e.* speciation) of soluble Fe, Co and Ni. The outcomes revealed that the speciation of soluble Fe was mainly controlled by the formation of Fe-sulfide and Fe-thiols complexes. Solubility of Co was likely regulated by the presence of compounds of microbial origin, whereas Ni was mainly co-precipitated and adsorbed onto FeS surfaces and precipitated as NiS in solid phase. The speciation of Ni in stillage-fed biogas tank reactors was also investigated by Gustavsson et al. (2013). They observed that precipitation of Ni was associated to acid volatile sulfides (AVS).

A dynamic mathematical model based on anaerobic digestion model no.1 (ADM1) was implemented by Maharaj et al. (2018) to assess the interaction of trace elements with inorganic species including S^{2-}, PO_4^{3-} and CO_3^{2-}. The results showed that trace elements mainly precipitated as sulfide species, whereas a small quantity of elements precipitated with carbonates at pH ranging from 6 to 8. Similarly, trace amount of microelements precipitated with phosphate during the simulation (Maharaj et al., 2018).

In addition, trace elements could be complexed with organic chelators becoming either more or less available for microbial uptake depending on the binding strength of metal-organic complexes. Such organic compounds contain functional groups such as carboxyl, hydroxyl or amino groups having high binding capacity to complex with trace elements (Callander and Barford, 1983). Gonzalez-Gil et al. (2003) observed that amino acids contained in yeast extracts form soluble complexes with Ni and Co which prevent their precipitation with sulfide, and consequently increase elements available for microorganisms. Moreover, extracellular polymeric substance (EPS) have different binding ability toward trace elements (D'Abzac et al., 2013, 2010) and they potentially control trace elements bio-availability. Other complexing agents capable of keeping trace elements in solution are ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA) and ethylenediamine-N,N′-disuccinic acid (EDDS). The latter chelating agent is more biodegradable and has a lower environmental risk compared to EDTA and NTA (Thanh et al., 2017; Zhang et al., 2015). These synthetic chelating agents are often supplied together with trace elements into anaerobic digesters to prevent trace elements precipitation with sulfides, and therefore enhance the bio-availability of trace elements to microorganisms (Thanh et al., 2017; Vintiloiu et al., 2013; Zhang et al., 2015).

In conclusion, the presence of organic and inorganic compounds deriving from the raw substrate as well as the microbial consortium and the operational conditions of anaerobic digestion processes (*e.g.* the neutral pH, the redox potential around -300 mV) will determine the speciation of trace elements in anaerobic digestion, and consequently in digestate (Möller and Müller, 2012). A better knowledge of the nature of organic ligands with

trace elements is an important point for determination of elements potentially available for uptake by microorganisms and plants when digestate is used as a soil amendment. However, after anaerobic digestion, the speciation of trace elements in digestate may change due to exposure to atmospheric air which may favor chemical oxidation of trace elements. Moreover, a re-distribution of trace elements between the liquid and solid phases in digestate may occur as showed in Figure 1. Current knowledge about the change in trace elements speciation in digestate exposed to air is very sparse.

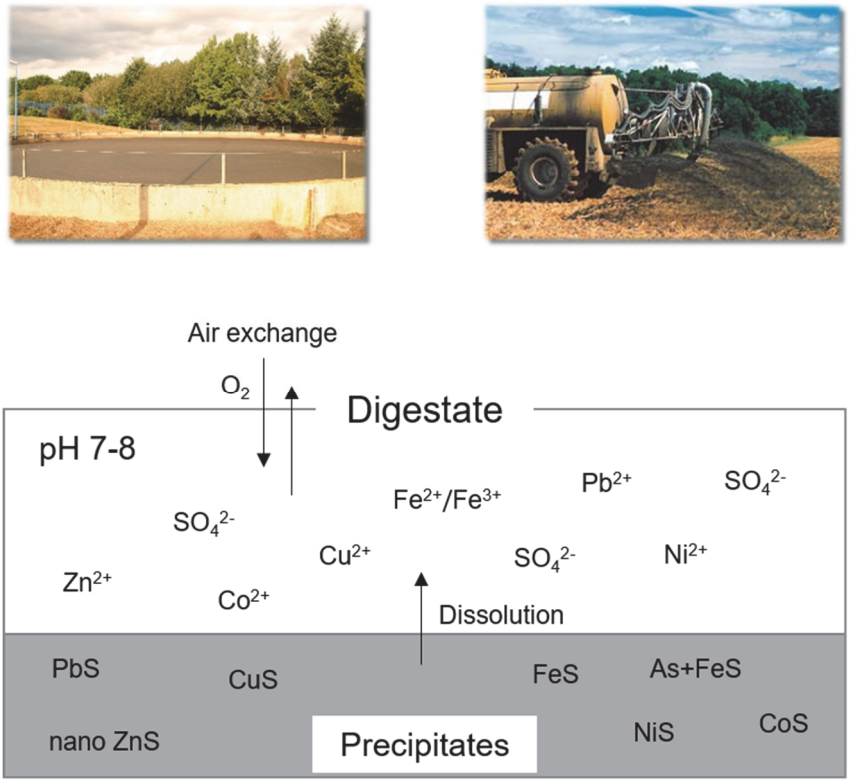


Figure 1. Potential changes of trace elements'speciation in digestate exposed to atmosphric air.

2.2 State of the art research on methods to assess bio-accessible trace elements

In this context, bio-accessibility refers to elements which are accessible for microbial uptake, for example they are not physically constrained in mineral or organic particles, whereas bio-availability refers to elements which can freely cross the organism's cellular membrane, and therefore they can influence their biological functions (Semple et al., 2004). As described in 2.1, speciation plays a key role influencing the availability of trace

elements for microbial uptake. Therefore, understanding speciation of trace elements in anaerobic digesters and in digestate is an important issue. Methods to assess trace elements speciation in anaerobic digester samples are extensively reviewed by van Hullebusch et al. (2016). These methods include solid phase S K-edge XANES combined with a thermodynamic equilibrium model to assess solid and liquid speciation of metal-sulfur compounds (Shakeri Yekta et al., 2014a), Cu K-edge XANES and Zn K-edge extended X-ray absorption fine structure spectroscopy (EXAFS) to assess solid speciation of Zn and Cu (Le Bars et al., 2018; Legros et al., 2017) which are also coupled to scanning electron microscopy (SEM-EDS) to get a qualitative characterization of the element's speciation (Formentini et al., 2017). Moreover, sequential extraction methods and biological measurements (*e.g.* uptake experiments by microorganisms or plants) could be mutually used to assess the bio-availability of trace elements (Harmsen, 2007). However, it should be highlighted that sequential extraction methods do not identify the speciation of trace elements (*e.g.* the isotopic composition, oxidation state, molecular structure) but rather separate trace elements according to their physical or chemical properties (*e.g.* strongly or weakly bound to carbonates, Fe and Mn oxides, and organic matter fractions) (van Hullebusch et al., 2016). Sequential extraction methods were developed to extract trace elements in fractions having different degree of mobility into the environment (Filgueiras et al., 2002). Therefore, reagents (*e.g.* un-buffered salts, weak acids, reducing and oxidising agents and strong acids (Filgueiras et al., 2002)) are applied in sequence to an aliquot of sample and the concentration of trace elements released in solution in each fraction is quantified by conventional analytical instruments such as inductively coupled plasma-optical emission spectrometry (ICP-OES) (Braga et al., 2017; Zhu et al., 2014). Ortner et al. (2014) applied the modified Tessier sequential extraction procedure to samples collected from industrial and agricultural anaerobic digestion plants. This method separates elements in the exchangeable fraction that is considered highly bio-available to microorganism, elements bound to carbonates, elements bound to organic matter and sulfides that have poor mobility and the residual fraction of trace elements. The authors identified that most of Fe was found in the residual and organic/sulfide fractions, whereas Co, Cu, Ni and Zn were mainly found the in water soluble and exchangeable fractions and therefore the elements were bio-accessible for microbial uptake during anaerobic digestion. The BCR sequential extraction method was applied on a substrate and digestate sample by Cestonaro do Amaral et al. (2014). Compared to the modified Tessier method, this extraction procedure involves the use of different reagents and separate trace elements in the exchangeable or bound to carbonates fraction, elements bound to hydrated oxides of Fe and Mn, elements linked to organic matter and sulfide and the residual fraction that is identified as stable fraction of elements. The authors found that the highest concentrations of Zn and Cu were bound to the organic matter sulfide fraction in both substrate and digestate. Therefore, these elements

are not immediately bio-accessible for microorganisms. However, the authors observed that a lower proportion of trace elements was bound to hydrated oxides of Fe and Mn in digestate compared to substrate and a higher proportion of CU and Zn was bound to organic/sulfides fraction in digestate than substrate, suggesting that the anaerobic digestion process enhances the formation of stable forms of Zn and Cu. Filgueiras et al. (2002) reviewed different sequential extraction schemes, including the Tessier and BCR methods, and highlighted a lack of uniformity in the procedures including the extracting reagents and the order of their application. In particular, the authors did not find agreement in procedures to target the fraction of trace elements associated with organic matter (Filgueiras et al., 2002). For instance, some authors proposed a prior decomposition of organic matter to facilitate the release of the following fractions while others employ different reagents such as sodium pyrophosphate and hydrogen peroxide to extract fractions associated with humic acid and residual organic matter and sulfides, respectively (Filgueiras et al., 2002). Moreover, no information was provided to differentiate the contribution of sulfides and organic matter phases to the released trace elements by hydrogen peroxide in anaerobically treated sludge (Braga et al., 2017; Zufiaurre et al., 1998). Therefore, an implementation of sequential extraction procedures is required to identify the contribution of organic matter to bind trace elements and their degree of bio-accessibility.

To overcome some limitations associated with sequential extraction procedures such as the poor selectivity of chemical reagents to target specific fractions of trace elements, the lack of uniformity in the procedures and possible changes in trace element speciation after reagents are added (Filgueiras et al., 2002; Shakeri Yekta et al., 2012). Thanh et al. (2016) identified DGT technique as a promising technique to determine bio-accessible metal concentrations in anaerobic bioreactors. This technique allows sampling labile trace elements after diffusion through a gel and accumulation on a binding gel in the DGT device during a known time period (Zhang and Davison, 2015). The labile elements comprise free ions and weakly bound inorganic and organic complexes, and thereby would represent the most readily bio-accessible species of trace elements (Zhang and Davison, 2015). Colloidal and particulate elements are excluded due to size restrictions of the diffusive gel (Zhang and Davison, 2015). DGT devices are applied in situ and do not require sample manipulation, preventing changes in trace elements speciation (Hooda et al., 1999). Moreover, this technique gives the possibility to simultaneously target several trace elements using a single binding gel. For examples binding gels containing Chelex-100 beads, a copolymer containing the iminodiacetic acid functional group, have high selectivity to chelate divalent and trivalent cations (Zhang and Davison, 1995), whereas binding gels made with zirconium oxide are used to sample oxyanion (Wang et al., 2016). After exposition of DGT devices to the sample medium, the binding

gel is recovered and eluted to release the sorbed trace elements which are quantified by conventional analytical instruments such as inductively coupled plasma-mass spectrometry (ICP-MS) (Bourven et al., 2017; Zhang and Davison, 1999). Finally, the original concentration of labile trace elements in the sample is back-calculated (Zhang and Davison, 1995).

The DGT technique was widely used in natural waters and soils to investigate the speciation of several trace elements and to assess their bio-accessibility (Zhang and Davison, 2015). Currently, data regarding the relationship between DGT-labile element concentrations and their bio-accessibility in digestate are very sparse. To our knowledge, Bourven et al. (2017) addressed this topic only for Cd during anaerobic digestion of whey in batch tests. The authors demonstrated DGT-labile Cd content contributed to the initial inhibition of biogas production and enzymatic activities (*i.e.* β-galactosidase and TTC-dehydrogenase). However, such correlation was absent after 21 days of anaerobic digestion. Moreover, Takashima et al. (2018) used DGT technique to measure labile Co and Ni species in digested sewage sludge filtrates. The authors showed that 70–88% of soluble Ni was DGT-labile, versus 5–10% of soluble Co in digested sludge filtrates, meaning that Ni was more bio-available than Co. Such studies demonstrate that DGT based fractionation can be used to predict bio-accessibility. However, no methodological development has been performed to adapt this technique to the digestate matrix. Moreover, the use of DGT is not straightforward in such complex matrix (*e.g.* multi-element contamination, high organic content) and requires preliminary validation or adaptation of the procedure for several trace elements in digestate.

3 Research Objectives and Questions

In relation to the methods and techniques currently used to assess bio-accessible trace elements, it is hypothesized that a single sequential extraction procedure could simultaneously predict bio-accessible organic matter and trace elements in substrate and digestate. Moreover, the potential association of trace elements with the extracted organic matter fractions is also investigated. Following the discussions on the limitations associated with the sequential extraction procedure such as possible changes in trace element fractionation caused by the sequential addition of chemical reagents and poor extraction efficiency for certain trace elements, it is assumed that DGT technique could increase the sensitivity of trace elements monitoring without affecting trace elements speciation. In fact, DGT technique allows in situ accumulation of trace elements. Accordingly, DGT technique could monitor the trend of labile trace elements over time in digestate exposed to air. Indeed, it was supposed that atmospheric exposure could impact trace elements distribution among labile, soluble and particulate fractions in digestate during storage in open thanks or handling before land spreading. Therefore, the research questions to answer are the following:

1. Can a single organic matter sequential extraction procedure predict both organic matter and trace elements bio-accessibility? To what extent is it possible to establish the association of trace elements with the extracted organic matter fractions?
2. Can NMR spectroscopy validate the nature of organic molecules extracted by the sequential extraction procedure?
3. Is DGT a sensitive technique to fractionate trace elements in the complex matrix of digestate?
4. Does the digestate matrix interfere with trace elements accumulation in DGT samplers?

5. Does DGT technique give relevant information on size fractionation of trace elements in a digestate?
6. What is the distribution of trace elements over time between soluble and labile fractions in aerated digestate?

The organic matter sequential extraction procedure was applied on a substrate and digestate sample collected from a full-scale anaerobic digestion plant. Questions 1 addresses the feasibility of the procedure to simultaneously assess bio-accessible organic matter and trace elements and to identify the interrelationship between these two parameters. Furthermore, the nature of the organic molecules extracted by the sequential extraction procedure were further explored by nuclear magnetic resonance (NMR) spectroscopy (question 2). The potential of DGT technique to fractionate trace elements was investigated in digested sewage sludge (question 3). Moreover, the possible organic matter interference on the estimation of labile elements' concentrations in digestate was studied (questions 4). Size fractionation of trace elements by using two different diffusive layers in DGT samplers was also evaluated (question 5). Finally, the effect of different rates of aeration on the mobility of trace elements (*i.e.* distribution between labile, soluble and particulate) was monitored over time by DGT technique in digested sewage sludge (question 6). The methodological approach to answering the research questions is presented in Figure 2.

Readers should note that, in this thesis, the word bio-accessible is preferred rather than bio-available since biological measurements are not combined with fractionation methods.

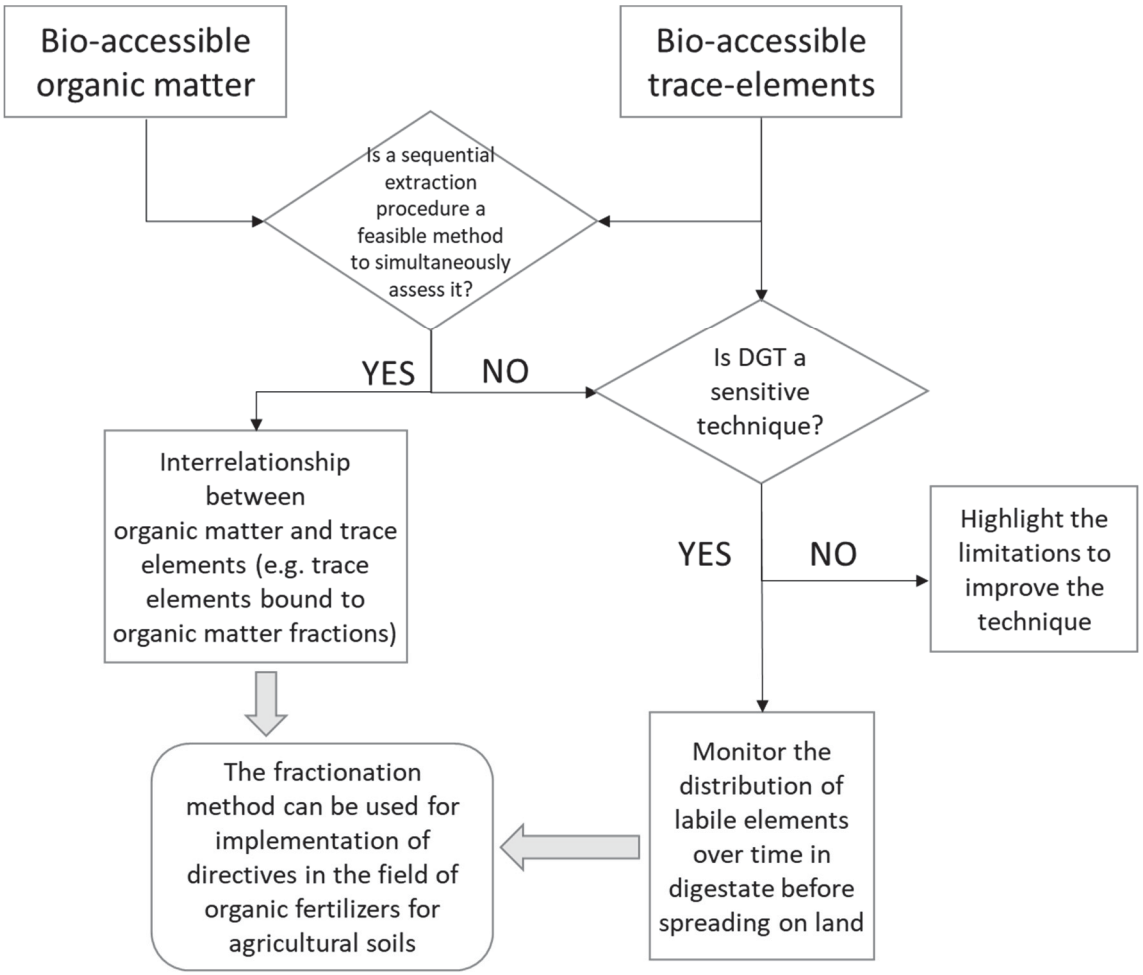


Figure 2. Logic chart representing the experimental strategy adopted to assess bio-eccessible trace elements and organic matter in digestate samples.

4 Summary

4.1 Methodology

4.1.1 Overview of the experiments

A sequential extraction procedure, adapted for organic matter fractionation, was implemented to simultaneously fractionate organic matter and trace elements for the assessment of the bio-accessibility of a combined source of carbon and micronutrient trace elements in substrate and digestate deriving from an anaerobic co-digestion plant (Paper I). The adopted organic matter sequential extraction procedure was developed by Jimenez et al. (2017, 2014) to assess the accessibility of organic matter as carbon and energy sources for microorganisms in anaerobic digesters. The method consists of treating samples with a series of reagents to extract organic matter fractions which are operationally defined from the most to the least soluble forms, representing high to low degree of bio-accessibility. For comprehensive description of the sequential extraction procedure, the reader can refer to paragraph 4.1.3. The liquid fractions recovered by the sequential extraction procedure, containing operationally defined organic matter fractions were analyzed to estimate dissolved organic carbon (C) and trace elements concentrations. Moreover, changes in structural characteristics of the solid residues collected after each step of the extraction procedure was analysed by nuclear magnetic resonance (NMR) spectroscopy in order to assess different organic groups in the samples, which were removed by reagents used during the sequential extraction procedure. All analytical procedures are described in section 4.1.6.

Following some limitations encountered during the sequential extraction procedure such as possible interactions between trace elements and the extracting reagents which may

generate analytical errors, sample contamination (*i.e.* elemental concentrations in some fractions < limit of detection or quantification) and matrix interference on the measured trace elements' concentrations due to the regents used during the extraction procedure, the bio-accessibility and mobility of trace elements (*i.e.* Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se and W) was investigated by the DGT technique in digested sewage sludge for the first time (Paper II). At first, the DGT deployment time was optimized by deploying DGT samplers from 4 hours to 9 days in digested sewage sludge. Moreover, the potential interference from the digestate matrix on the DGT samplers' performance was evaluated. The experimental work is described in sections 4.1.4.2 and 4.1.4.3. To further understand about size fractionation of trace elements, a simultaneous deployment of DGT samplers equipped with restricted (pore size < 1nm) and standard diffusive gels (pore size > 5 nm) was investigated.

The DGT technique was further employed to investigate the mobility and distribution trend of trace elements in the digestate exposed to air (Paper III). It is hypothesized that the distribution of labile, soluble and particulate trace elements may change over time under oxidizing conditions similarly to digestate storage in open tanks and handling before spreading on land. Therefore, digested sewage sludge was kept open to air in the laboratory to promote oxidation of the sample during 10 weeks, assuming that the experimental work could mimic digestate oxidation from air during storage in open tanks. Subsequently, aeration was enhanced during 2 supplementary weeks, assuming that forced aeration could mimic handling before spreading the digestate on land. Details of the experimental set up are described in 4.1.4.4.

The overall experimental plan of this research is summarized in Figure 3.

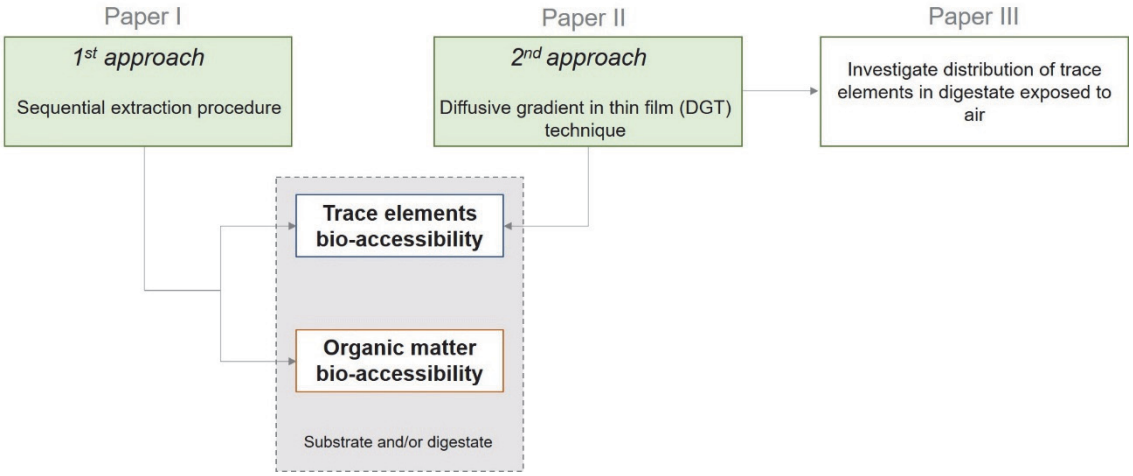


Figure 3. Overview of experiments carried out in this research work.

4.1.2 Samples

The organic matter sequential extraction procedure was applied on substrate and digestate collected from a full-scale anaerobic co-digestion plant located in Linköping, Sweden. The co-digestion plant treats the organic fraction in household waste, slaughterhouse and industrial waste in a continuous flow-stirred reactor tank at 42°C. The substrate was collected from the tank after 1-hour pasteurization at 70°C and trace elements addition, whereas the digestate was collected from the main anaerobic digester sampling port. The sample composition (*e.g.* pH, total and volatile solids and total elemental content) is reported in Table 1. The samples were collected in acid washed polypropylene (PP) bottles flushed with nitrogen (N_2) prior to sampling and closed with a lid after collection to reduce sample exposure to atmospheric air during sampling and transportation from the plant to the laboratory.

The DGT technique was applied on digested sewage sludge collected from a municipal waste-water treatment plant in Limoges, France. The anaerobic digester treats activated sludge at mesophilic temperature. For each experiment (*i.e.* Papers II and III), between 18 L and 20 L of sample was collected directly from a pipe before discharge in an open storage tank. The sample was collected in PP tanks up to maximum capacity and closed with a lid to limit sample exposure to open air. Once in the laboratory, the sample was stored at 4°C for less than 24 hours before starting the experiments. The samples composition is also reported in Table 1.

Table 1. Chemical composition of the samples used for the experimental works. When applicable, total element content, total and volatile solids (TS and VS) and pH are mean of duplicates or triplicates ± standard deviation.

	Paper I		Paper II	Paper III
	Substrate	Digestate	Digested sewage sludge	Digested sewage sludge
pH	4.9	8.1	7.3	7.8 ± 0.3
TS (%)	14.6 ± 0.1	4.8 ± 0.2	3.3 ± 0.0	3.8 ± 1.6
VS ($\%_{TS}$)	91.4 ± 0.3	76.2 ± 0.6	69.3 ± 0.2	63.9 ± 1.3
Al ($\mu g/g_{TSin}$)	722 ± 60	1359 ± 34	13311 ± 778	9070 ± 1420
As ($\mu g/g_{TSin}$)	0.22 ± 0.02	1.5 ± 0.1	100 ± 6	123 ± 5

Cd (µg/g_{TSin})	0.10 ± 0.00	0.22 ± 0.01	1.94 ± 0.13	1 ± 1
Co (µg/g_{TSin})	4.04 ± 0.04	10.5 ± 0.1	7 ± 1	6 ± 1
Cr (µg/g_{TSin})	2.0 ± 0.1	5.9 ± 0.2	72 ± 4	35 ± 1
Cu (µg/g_{TSin})	<35.8‡	40.4 ± 6.4	449 ± 24	334 ± 10
Fe (µg/g_{TSin})	4393 ± 71	12623 ± 223	57006 ± 5449	61343 ± 405
Mn (µg/g_{TSin})	46.1 ± 1.5	121 ± 5	601 ± 42	733 ± 22
Mo (µg/g_{TSin})	0.68 ± 0.02	2.3 ± 0.1	7.1 ± 0.3	5 ± 1
Ni (µg/g_{TSin})	2.7 ± 0.1	23.5 ± 0.2	37 ± 8	<19*
Pb (µg/g_{TSin})	<1.8‡	3.8 ± 0.6	89 ± 9	62 ± 1
Sb (µg/g_{TSin})	n.a.	n.a.	n.a.	<3*
Se (µg/g_{TSin})	n.a.	n.a.	<18§	<12#
W (µg/g_{TSin})	n.a.	n.a.	n.a.	3 ± 1
Zn (µg/g_{TSin})	68.1 ± 0.6	168 ± 7	n.a.	n.a.

‡Method Limit of Quantification (MLQ)=average blanks ± 10*standard deviation blanks (n=3), using 0.09 L/$g_{TSinitial}$ as conversion factor.

*MLQ=average blanks ± 10*standard deviation blanks (n=18), using 0.29 L/$g_{TSinitial}$ as conversion factor.

§MLQ=average blanks ± 10*standard deviation blanks (n=10), using 0.35 L/$g_{TSinitial}$ as conversion factor.

#Method Limit of Detection (MLD)=average blanks ± 3*standard deviation blanks (n=18), using 0.29 L/$g_{TSinitial}$ as conversion factor.

n.a.=not available

4.1.3 Organic matter sequential extraction procedure

Organic matter and trace elements bio-accessibility was assessed by implementing a sequential extraction procedure originally designed for organic matter fractionation (Jimenez et al., 2017, 2014). The sequential extractions of dissolved organic matter

(DOM), readily extractable organic matter (REOM) and slowly extractable organic matter (SEOM) fractions were carried out according to Jimenez et al. (2014), whereas extraction of extractable soluble from particulate organic matter (SPOM) and poorly extractable organic matter (PEOM) fractions were performed according to Jimenez et al. (2017). The latter modified protocol includes calcium chloride ($CaCl_2$) reagent for SPOM extraction and sulfuric acid (H_2SO_4) for PEOM extraction compared to the procedure proposed by Jimenez et al. (2014). Moreover, some modifications were included in the protocol to adapt the method for simultaneous extraction of organic matter and trace elements (Table 2). The main modifications involve the use of raw sample, rather than freeze dried sample, and N_2 flushing during operations to reduce sample oxidation and changes in trace element speciation (e.g. formation of metal oxides) which would determine a change in the bio-accessibility pattern of trace elements. Moreover, the sample mass and the volume of reagents were decreased compared to the original procedures to adapt the method to facilities available in the laboratory such as the high speed centrifuge (Beckman J2-21M, USA), which was used to separate the supernatants from the solids.

In short, the first step of the procedure separates DOM from the solid residue and was performed immediately after sample transportation to the laboratory to avoid changes in partitioning of trace elements between liquid and solid phase. Approximately 300-600 mL of sample, whit a total solid content of 4.8±0.2 wt% and 14.6±0.1 wt% for digestate and substrate, respectively, was centrifuged at 18600×g for 30 min at 10°C. Then, the supernatant containing DOM was filtered through 0.45 µm polyethersulfone (PES) syringe filters (Pall Laboratory). The solid residue was flushed with N_2, sealed and stored at 4°C in PP centrifuge tubes (Sarstedt) before performing the next extraction step. In the second step, SPOM was extracted according to the procedure of Jimenez et al. (2017). Approximately 3 g of pellet were shaken in polypropylene copolymer (PPCO) tubes (Thermo Scientific Nalgene) with 24 mL (mass ratio 1:8) of 10 mM $CaCl_2$ (pH 8) at 200 rpm and 30°C for 15 min. The suspension was then centrifuged at 18600×g for 30 min at 4°C and the supernatant containing SPOM was recovered and filtered through 0.45 µm PES syringe filters. The residual solid was treated with the same reagent three more times. During extraction of SPOM, N_2 was flushed in the tubes.

Subsequently, the solid residue was rinsed four times with 24 mL of 10 mM NaCl and 10 mM NaOH (pH 11) (Jimenez et al., 2014). The suspension was shaken, centrifuged and filtered to recover REOM fraction. Thereafter, the residual pellet was used to extract carbonate, sulfides and hydroxides (CSH) fraction by adding 24 ml of 0.1 M HCl for 1 h at 30°C and 200 rpm (Jimenez et al., 2014). Unlike the original procedure (Jimenez et al., 2014), this fraction was recovered for further analyses. The resulting solid residue was washed with ultrapure water and neutralized to pH 7. Subsequently, the solid residue

was suspended in 24 ml of 0.1 M NaOH (pH 12) and shaken at 200 rpm and at 30°C for 1 h to recover the SEOM fraction (Jimenez et al., 2014). This step was repeated three more times. Finally, the residual pellet was shaken two times with 24 mL of 72% (w:w) H_2SO_4 for 3 h at 30°C and 200 rpm for extraction of PEOM (Jimenez et al., 2017). The residual solid, which is the non-extractable organic matter (NEOM), was recovered and freeze-dried for further analyses. The sequential extraction procedure was performed on triplicate samples. All reagents were prepared in acid washed glassware and with ultrapure deaerated water. All chemical reagents were of analytical grade.

During the sequential extraction procedure, procedural blanks were treated along with the substrate and digestate samples. Therefore, 36 and 96 procedural blanks were collected for trace elements and dissolved organic carbon analysis, respectively.

Table 2. Sequential extraction procedure adapted from Jimenez et al. (2017, 2014) with some modifications highlighted in italic font. The extracted fractions are listed in order of decreasing bio-accessibility.

Organic Matter Fraction	Reagent	Extraction Method	Bio-accessibility Degree
DOM	-	Centrifugation (18600g, 30 min, *10°C*), filtration 0.45 µm, *N_2 flushing*	High
SPOM	*24 mL* of 10 mM $CaCl_2$	4 × shaking (*200 rpm*, 30°C, 15 min), centrifugation, filtration, *N_2 flushing*	
REOM	*24 mL* of 10 mM NaCl + 10 mM NaOH	4 × shaking (200 rpm, 30°C, 15 min), centrifugation, filtration, *N_2 flushing*	
CSH	*24 mL* of 0.1 M HCl + ultrapure water rinsing	1 × shaking (200 rpm, 30°C, 60 min), centrifugation, filtration, *N_2 flushing*	
SEOM	*24 mL* of 0.1 M NaOH	4 × shaking (200 rpm, 30°C, 60 min), centrifugation, filtration, N_2 flushing	
PEOM	*24 mL* of 72% H_2SO_4	2 × shaking (*200 rpm*, 30°C, 3 h), centrifugation, filtration, *N_2 flushing*	Low

4.1.4 DGT experimental set-up

DGT-labile trace elements (*i.e.* the most readily bio-accessible form of trace elements (Zhang and Davison, 2015)) were sampled by Chelex-DGT samplers for cationic species (Al, Cd, Co, Cr (III), Cu, Fe, Mn, Ni and Pb) and zirconia-DGT samplers (Zr-DGTs) for anionic species (As, Mo, Sb, Se and W). The selectivity of Chelex-DGT sampler over the oxidation state of Cr species was previously demonstrated by Ernstberger et al. (2002). Each DGT consisted of a binding gel (Chelex or Zr), a diffusive gel and a filter membrane

enclosed in a piston type holder as showed in Figure 4. The preparation of DGT samplers for different experiments is described below.

Blank DGT devices were also prepared in duplicate and treated alongside exposed devices according to the deployment time of the experiment. The blanks were stored in a moistened plastic bag to keep a sufficient humidity for the gels and disassembled alongside the other samplers.

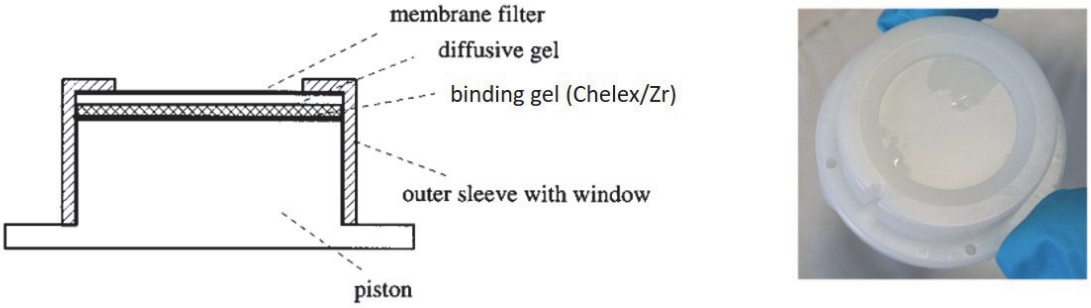


Figure 4. On the left a schematic representation of DGT sampler adapted from Zhang et al. (1998). On the right an image of assembled DGT sampler.

4.1.4.1 DGT preparation

Chelex binding gels were prepared according to the procedure described by Zhang et al. (1998), whereas Zr binding gels were made according to Devillers et al. (2016). Unless stated otherwise, DGT samplers were equipped with standard polyacrylamide gels (15% acrylamide and 0.3% agarose-derived cross linker, 0.77 mm thick) prepared according to Zhang et al. (1998) (Paper II and III). In addition, restricted diffusive gels (15% acrylamide and 0.75% bisacrylamide cross linker, 0.75 mm thick) with pore size <1 nm (Zhang and Davison, 1999) were investigated (Paper II). The latter gels were prepared following a procedure slightly modified from Scally et al. (2006), that is polymerization was performed by mixing 200 µL of 10% (m/V) freshly prepared ammonium persulfate (Fisher Scientific) and 8 µL of tetramethylethylenediamine (TEMED) (Aldrich) with 10 mL of gel solution (15% acrylamide and 0.75% bisacrylamide cross linker). To cast the gel, two glass plates were separated by a 0.75 mm thick Teflon spacer and the gel solution was poured between the plates. The plates were placed in an oven at 45°C for 45 min to allow a rapid polymerization. Compared to a standard diffusive gel, the restricted gel is brittle. Consequently, the gel plates were cut into round disks and rinsed with ultrapure water at least five times during 24 hours to remove any impurities from the gels. The diffusive gels were then stored in 0.01 M $NaNO_3$ at 4°C.

A filter membrane of 0.4 µm pore size in polycarbonate or 0.2 µm pore size cellulose acetate membrane was placed on the top of the diffusive gel.

4.1.4.2 DGT deployment time in digestate matrix

The optimization of DGT samplers' deployment time was achieved by performing two experiments 1) a "short term" experiment to validate the establishment of steady state conditions in the samplers, and 2) a "long term" experiment to increase the sensitivity of the method. Therefore, triplicate devices of both Chelex and Zr were immersed for 4, 8,18 and 24 h ("short term" experiment) and for 24, 48, 72, 144 and 216 h ("long term" experiment) in digested sewage sludge which was kept under anaerobic conditions by covering its surface with paraffin oil and a plastic film (Figure 5). To note that, before starting the experiment, the devices were immersed overnight in N_2 flushed ultrapure water to remove oxygen from them.

The temperature was recorded every 10 min by a Tinytag data logger (TG-4100, Gemini Data Loggers, UK) in the sample.

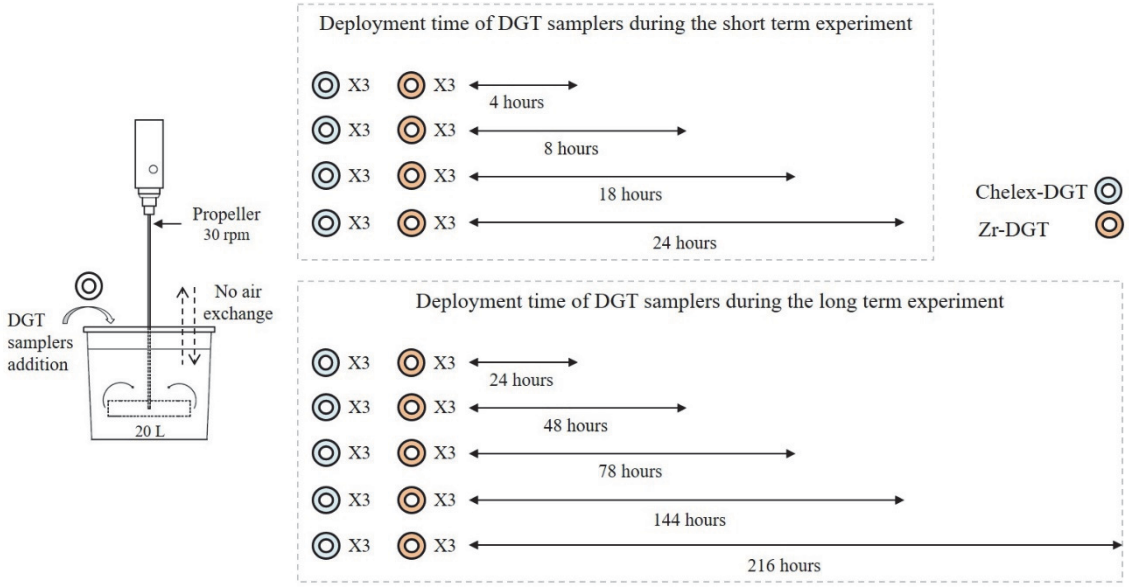


Figure 5. On the left, the pilot scale tank containing the digested sludge. On the right, a scheme for the deployment time of DGT samplers (in triplicate).

4.1.4.3 Diffusive gel loading in a digestate matrix

The potential interference from the digestate matrix on the diffusion and accumulation of trace elements in the binding gels was investigated by deploying triplicate Chelex and Zr-DGT samplers in digested sewage sludge for 24 hours to load their diffusive gels with

the digestate matrix (Paper II). “Soiled” DGT samplers were built with the pre-exposed diffusive gels and new Chelex and Zr binding gels. Additionally, triplicate DGT samplers were built with new diffusive and binding gels as controls in the experiment. Both samplers (control and soiled) were immersed in 1.5 L of 10^{-2} M NaCl solution spiked with cationic (Cd (II), Co(II), Cu (II), Ni (II) and Pb (II)) or anionic elements (As (III), Mo (VI) and Se (IV)) for 4 h under continuous stirring. The concentrations of the elements spiked in the solution and the conditions of the experiments (pH and temperature) are summarized in Appendix in Table A1.

To check the contamination of the binding gel brought by the “soiled” diffusive gel, three blank DGT samplers were built with “soiled” diffusive gels and new Chelex and Zr binding gels.

4.1.4.4 Distribution of trace elements in digestate exposed to air

DGT-based fractionation procedure was used to assess the potential impact of digestate aeration on trace elements mobility and bio-accessibility (Paper III). Therefore, about 18 L of digested sludge were kept into a laboratory-scale PP tank in aerobic condition under a fume hood. The digested sludge was continuously stirred with an overhead plastic propeller at 30 rpm to control the experimental conditions and to allow air transfer within the sample. A Tinytag data logger (TG-4100, Gemini Data Loggers, UK) was used to record the temperature in the sample every 10 min.

After 10 weeks, the surface to volume ratio varied from 0.39 dm^{-1} (7.1 dm^2:18 L) to 0.51 dm^{-1} (7.1 dm^2:14 L) because of multiple sample collection (*i.e.* 90 mL of sample in duplicate retrieved each sampling time for the analyses described in 4.1.6.3). Therefore, passive aeration was progressively favored while the experiment continued. Then, aeration was enhanced during 2 supplementary weeks by introducing 4 micro-bubble air diffusers in the digested sludge. All diffusers were connected to air pumps (Newair or Optima) having airflow rates from 60 to 200 L/h.

DGT samplers, composed either of Chelex or Zr, were deployed for 24h in the digested sludge. The sequence of DGT sampler’s collection from the sludge is showed in Figure 6. Moreover, particulate and soluble trace elements were quantified during the experiment as described in 4.1.5.2.

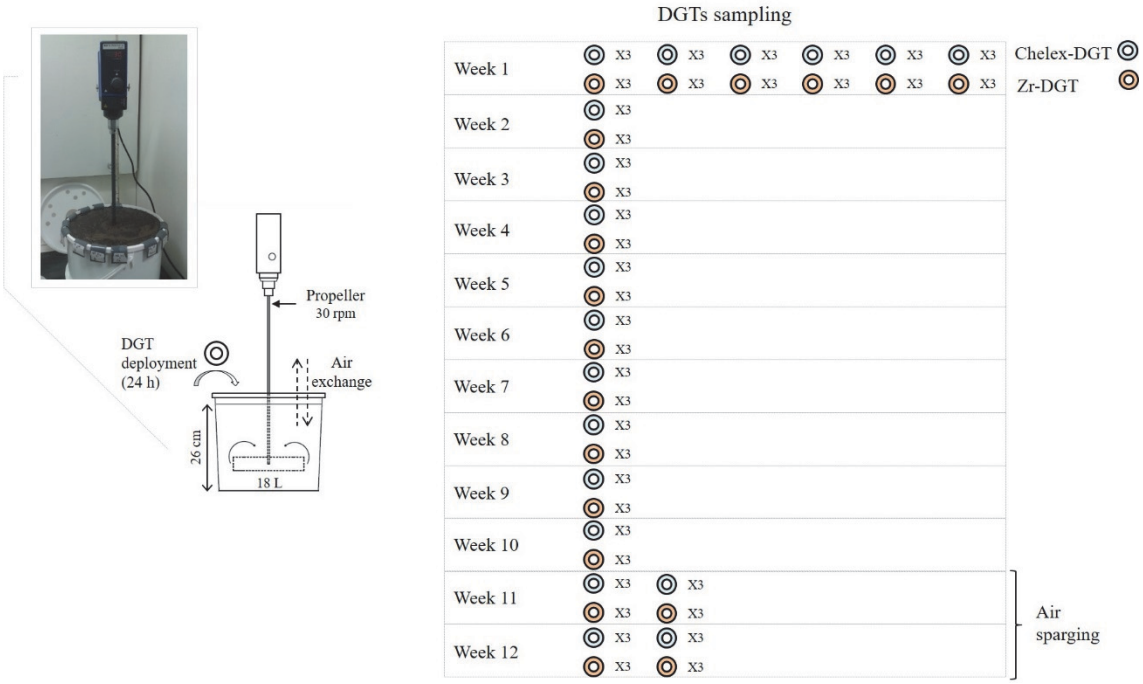


Figure 6. On the left, the pilot scale tank containing the digested sludge. On the right, a scheme of DGTs sampling throughout the experiment of digestate exposed to air.

After DGTs' retrieval, dissolved O_2, redox potential (E_h), pH. total and volatile solids (TS and VS), total and volatile suspended solids (TSS and VSS) and sulfate (SO_4^{2-}) concentration were monitored.

4.1.5 Calculations

4.1.5.1 DGT labile concentration

After retrieval from the digested sludge, DGT samplers were rinsed with ultrapure water and disassembled to recover the binding gels. The accumulated mass (m) of trace elements in each DGT sampler was determined after elution of the binding gel. The Chelex binding gels were eluted in 2 mL of 1 M HNO_3 for 24 hours and the Zr binding gels in 2 mL of $5 \cdot 10^{-3}$ M NaOH and 0.5 M H_2O_2 for 24 hours at room temperature (20 ± 1 °C). The concentration of trace elements in the eluents (C_e) were quantified by inductively coupled plasma mass spectrometry (ICP-MS) or microwave plasma atomic emission spectrometer (MP-AES) (see section 4.1.6). The accumulated mass is determined according to equation (1):

$$m = \frac{C_e \times V_e}{f_e} \quad , \qquad \text{Eq. (1)}$$

where V_e is the volume of the eluents (2 mL) and f_e is the elution factor (values are reported in Appendix in Table A2).

The concentration of labile trace elements, C_{DGT}, in the sample is then derived using equation (2) based on Fick's first law (Zhang and Davison, 1995):

$$C_{DGT} = \frac{m \times \Delta_{MDL}}{\mathrm{D} \times \mathrm{t} \times \mathrm{A}}, \qquad \text{Eq. (2)}$$

where Δ_{MDL} is the thickness of the material diffusion layer (*i.e.* diffusive gel plus membrane), t is the time of DGT sampler's exposure in the sludge, D is the coefficient of diffusion of the specific element in the diffusion layer and A is the geometric area of the DGT holder window (3.14 cm^2). The values of D for a standard diffusive gel are taken from literature (Table A3) and corrected for the average temperature (T) recorded every 10 min by a Tinytag data logger during each deployment using Stokes–Einstein relation (Zhang and Davison, 1999) as follows:

$$\frac{D_1 \times \eta_1}{T_1} = \frac{D_2 \times \eta_2}{T_2}, \qquad \text{Eq. (3)}$$

where η is the viscosity of the water taken from the NIST chemistry WebBook (Lemmon et al., 2010). The D values for the restricted gel are equal to 70% of the D for a standard gel, based on the work of Scally et al. (2006) and Shiva et al. (2015) as summarized in Appendix in Table A4.

4.1.5.2 Fractionation procedure

Together with DGT-labile elements, particulate and soluble trace elements were monitored (Paper III). Particulate elemental concentration was calculated by subtracting the soluble to the initial total elemental content as presented in Figure 7. The procedure to estimate total and soluble elemental concentration is described in 4.1.6.

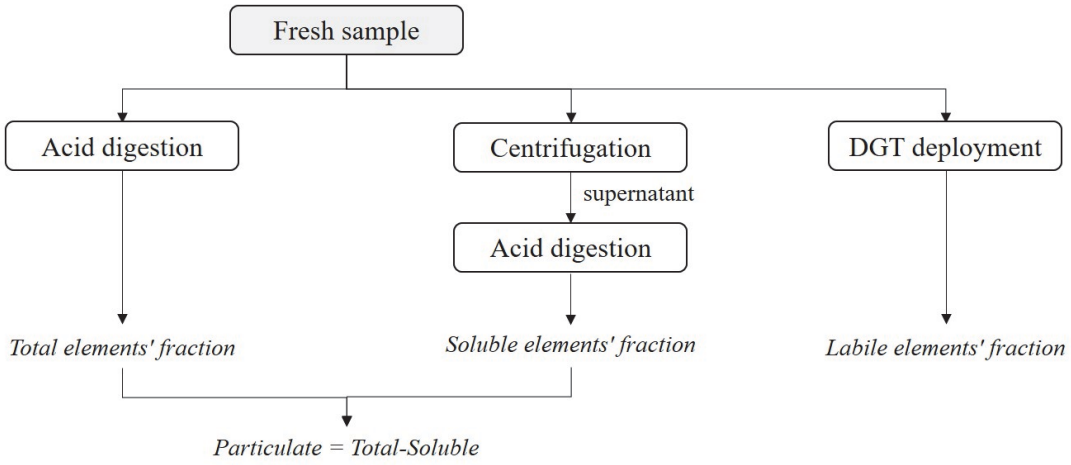


Figure 7. Fractionation procedure adopted in Paper III to estimate total, soluble, particulate and labile elements' fractions.

4.1.5.3 Data treatment

For the statistical analysis of results, a F-test was performed using Microsoft Excel 2013 to determine the variances of two sets of samples, then the two-tailed t-test was applied at 95% confidence interval.

4.1.6 Analytical procedures

4.1.6.1 Trace elements analysis

The total elemental concentration was estimated after acid digestion of the raw samples. Two procedures were followed. One procedure consists in diluting 5 g of raw sample with 6 mL of 69% HNO_3 and 3 mL of 37% HCl in a microwave oven (Multiwave GO, Anton Paar GmbH) at 180°C for 60 min (Papers II and III). The second procedure follows the Swedish standard method (SS028311) using 0.2 g dry mass of samples and 7 M HNO_3 in an autoclave at 120°C for 30 min (Paper I). All chemical reagents used were of analytical grade.

Soluble elemental concentration was determined from the supernatant recovered after centrifugation of raw sample at 3.000×g for 20 min. Then 2 mL of supernatant was acid digested following the first procedure described above (Papers II and III). After acid digestion, the samples were further diluted with ultrapure water.

Trace element concentration in the samples collected after the acid digestion method, the sequential extraction procedure or elution of DGT binding gels, was quantified by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700X in Papers II and

III or ICP-MS, Nexion 300D in Paper I). Moreover, Fe was analyzed by MP-AES (Agilent 4210) as reported in Papers II and III.

Blanks (*i.e.* ultrapure water adjusted to 2% HNO_3) and quality controls were analyzed to check for possible sample contamination and the performance of trace elements analysis, respectively.

4.1.6.2 NMR spectroscopy

The structural composition of organic molecules in the solid residues recovered after each step of the organic matter sequential extraction procedure was provided by NMR spectroscopy (Paper I). Solid residues were preferred to the liquid fractions to reduce possible interferences, generated by the chemical reagents, with the sample NMR signals. Before analysis, about 0.4 g dry mass of sample (solid residues) was pre-treated with 2 M HCl for 1 h to remove the paramagnetic trace elements that would be detrimental to the quality of the NMR spectra (Shakeri Yekta et al., 2018). The suspension was centrifuged and the supernatant was discarded, while the solid residue was recovered and freeze-dried. Approximately 80 mg of freeze-dried sample was transferred to 4 mm ZrO_2 rotors for solid state cross polarization magic angle spinning (CPMAS) ^{13}C NMR analysis. Approximately 100 mg of sample was milled using a Fritsch Pulverisette 7 planetary ball-mill for solution-state 1D ^{1}H and 2D ^{1}H-^{13}C heteronuclear single quantum coherence (HSQC) NMR analysis. The protocol used for grinding consisted of 5 × 10 min milling with 5 min pause in between to prevent overheating the samples. 20 mg of milled sample were transferred to 5 mm NMR tubes and 600 µL of deuterated dimethyl sulfoxide (DMSO-d_6) was added. The CPMAS ^{13}C analysis was performed in triplicate and these were later pooled to get sufficient material for liquid state NMR analysis.

Solid state CPMAS ^{13}C NMR spectra and liquid state 1D ^{1}H and 2D ^{1}H-^{13}C HSQC NMR spectra were acquired using a Bruker 500 MHz AVANCE III spectrometer equipped with a 4 mm MAS probe and a Bruker 600 MHz AVANCE III HD spectrometer equipped with a 5 mm cryoprobe, respectively. Spectra processing was performed in Topspin 3.5 (Bruker Biospin, Germany) and spectra were calibrated using adamantane as an external reference for CPMAS spectra or the residual DMSO peak ($\delta_{H/C}$: 2.49/39.5 ppm) in the case of 2D ^{1}H-^{13}C HSQC spectra.

The acquisition parameters for NMR analysis are reported in Table A5.

4.1.6.3 Physicochemical analysis

The analytical instruments and the methods to measure pH, Eh, dissolved oxygen, SO_4^{2-} concentration, TS, VS, TSS, VSS, dissolved organic carbon and total carbon and nitrogen content are listed in Table 3. For comprehensive description of the methods, the reader is referred to Papers I-III.

Table 3. Physichochemical analyses and analytical instruments used during the experiments in Papers I, II and III.

Parameter	Analytical technique and instrument	Reference
pH	Mettler Toledo pH electrode	Papers II, III
	InoLab 7310, WTW, pH meter	Paper I
Eh	Radiometer electrode	Paper III
Dissolved oxygen	ProODO™ optical sensor (YSI)	Paper III
Temperature	Tinytag data logger (TG-4100, Gemini Data Loggers)	Papers II, III
SO_4^{2-}	Turbidimetric method, sulfate test kit (SulfaVer 4 Method, HACH), spectrophotometer (DR 1900, HACH LANGE) at 450 nm	Paper III
TS, VS	French standard AFNOR NF T90-105 method	Papers II, III
	Swedish Standard method (SS-028113; 25)	Paper I
TSS, VSS	French standard AFNOR NF T90-105 method	Papers II, III
Dissolved organic carbon	Total organic carbon analyzer (TOC-VCHS, Shimadzu)	Paper I
Total carbon and nitrogen content	Combustion, CHNS/O elemental analyzer (EA2400, Perkin Elmer)	Paper I

4.1.7 Method limits of detection

The method's limits of detection were determined for each procedure (*i.e.* sequential extraction or DGT handling) to account for sample contamination. During the sequential extraction procedure, procedural blanks in triplicate were treated along with substrate and digestate samples (cf. section 4.1.3). Blank DGT devices were prepared in duplicate and treated alongside exposed devices during the experiments described in section 4.1.4. Moreover, for the acid digestion procedure to estimate total elemental concentration, ultrapure water blanks were treated alongside samples as described in 4.1.6.1. The method's limit of detection (MLD) and quantification (MLQ) were calculated according to IUPAC (Mocak et al., 1997) as the average plus three or ten times the standard deviation of the blanks for MLD and MLQ, respectively.

4.2 Results and Discussions

4.2.1 Simultaneous assessment of organic matter and trace elements' bio-accessibility by a sequential extraction procedure

The sequential extraction procedure was originally developed to predict bio-accessible organic matter as carbon and energy sources for microorganisms in anaerobic digesters (Jimenez et al., 2017, 2014). The novelty of this study is to evaluate the relevance of this sequential extraction procedure to simultaneously assess trace elements and organic matter fractions bio-accessibility in a substrate and digestate (Paper I). Moreover, the potential association of trace elements with the extracted organic matter fractions is evaluated. Indeed, to the best of the author's knowledge, no research work has been attempted to simultaneously assess bio-accessible organic matter and trace elements and the interrelations between these two using a single extraction procedure. Some limitations were identified and they are described in the following paragraphs.

4.2.1.1 Organic matter fractionation

First of all, results showed a different organic matter composition of substrate and digestate. Figure 8a and 8b present the distribution of organic carbon among the operationally defined organic matter fractions (*i.e.* DOM, SPOM, REOM, SEOM and PEOM), Figure 8c to 8f show the main organic groups by 2D ^{1}H-^{13}C HSQC NMR spectra and ^{13}C CPMAS NMR spectra in DOM solid residue from substrate and digestate. ^{13}C CPMAS NMR spectra (Figure 8e and 8f) are divided in five regions corresponding to different organic structures: aliphatic chains C (δ_C 0-47 ppm), carbohydrates (δ_C 47-90 ppm), anomeric C (δ_C

90-110 ppm), aromatic C (δ_C 110-160 ppm) and carbonyl C (δ_C 160-187 ppm) (Kögel-Knabner, 1997; Tambone et al., 2009).

Figure 8a shows that a large proportion of organic carbon in the substrate is present as DOM (76% of extracted organic C), whereas 15% of extracted organic C is present in SPOM, REOM and SEOM and only 9% of extracted organic C is present in PEOM. In digestate (Figure 8b), PEOM has the highest proportion of C among the organic matter fractions (47% of extracted organic carbon), whereas only 28% of extracted organic C is contained in DOM and 25% of extracted organic C is present in SPOM, REOM and SEOM. According to Jimenez at al. (2017, 2014), DOM fraction mainly contains water-soluble organic substances, whereas PEOM contains recalcitrant and insoluble organic compounds. Instead, SPOM, REOM and SEOM mainly contain proteins and sugars, lipids, humic-like and fulvic acid-like structures based on fluorescence spectroscopic characterization of the organic matter extracted in the supernatant after each step by Jimenez et al. (2017). To support the organic matter characterization of the extracted fractions provided by Jimenez et al. (2017, 2014), NMR spectroscopy was applied to identify the nature of the organic molecules in the solid residues recovered after each extraction step of the sequential extraction procedure.

The ^{13}C CPMAS NMR spectra acquired from substrate and digestate samples (Figures 8e and 8f) show a higher contribution of aliphatic, aromatic and carbonyl C resonances in spectra of the digestate compared to the substrate, while carbohydrate signals have a higher contribution in the substrate spectra. Accordingly, aliphatic, aromatic and carbonyl C, mainly attributed to lipid- and/or protein-like structures (Keeler et al., 2006; Kögel-Knabner, 1997; Simpson et al., 2011) are enriched in the solid phase of the digestate upon anaerobic digestion, suggesting that these molecules are chemically recalcitrant to the anaerobic digestion process.

Additionally, 2D ^{1}H-^{13}C HSQC NMR spectra from substrate (Figure 8c) and digestate (Figure 8d) show that anomeric signals at δ_H 4.45-5.2 and δ_C 99-102 ppm (Simpson et al., 2011) as well as O-alkyl signals at δ_H 3.4-3.8 ppm and δ_C 68-79 ppm (Soucémarianadin et al., 2017) from hemicellulose and starch are more readily degraded during the anaerobic digestion process compared to non-anomeric signals at δ_H 3.24-3.63 ppm and δ_C 60-72 ppm (Soucémarianadin et al., 2017) and anomeric C signal at $\delta_{H/C}$: 4.32/102.4 ppm (Soucémarianadin et al., 2017) from cellulose, which are left in the digestate. Interestingly, the peak from unsaturated double bonds of aliphatic structures such as fatty acids ($\delta_{H/C}$: 5.32/130 ppm, Figure 8c) is not visible in the digestate sample, indicating that aliphatic double bonds in the substrate are susceptible to degradation during the anaerobic digestion.

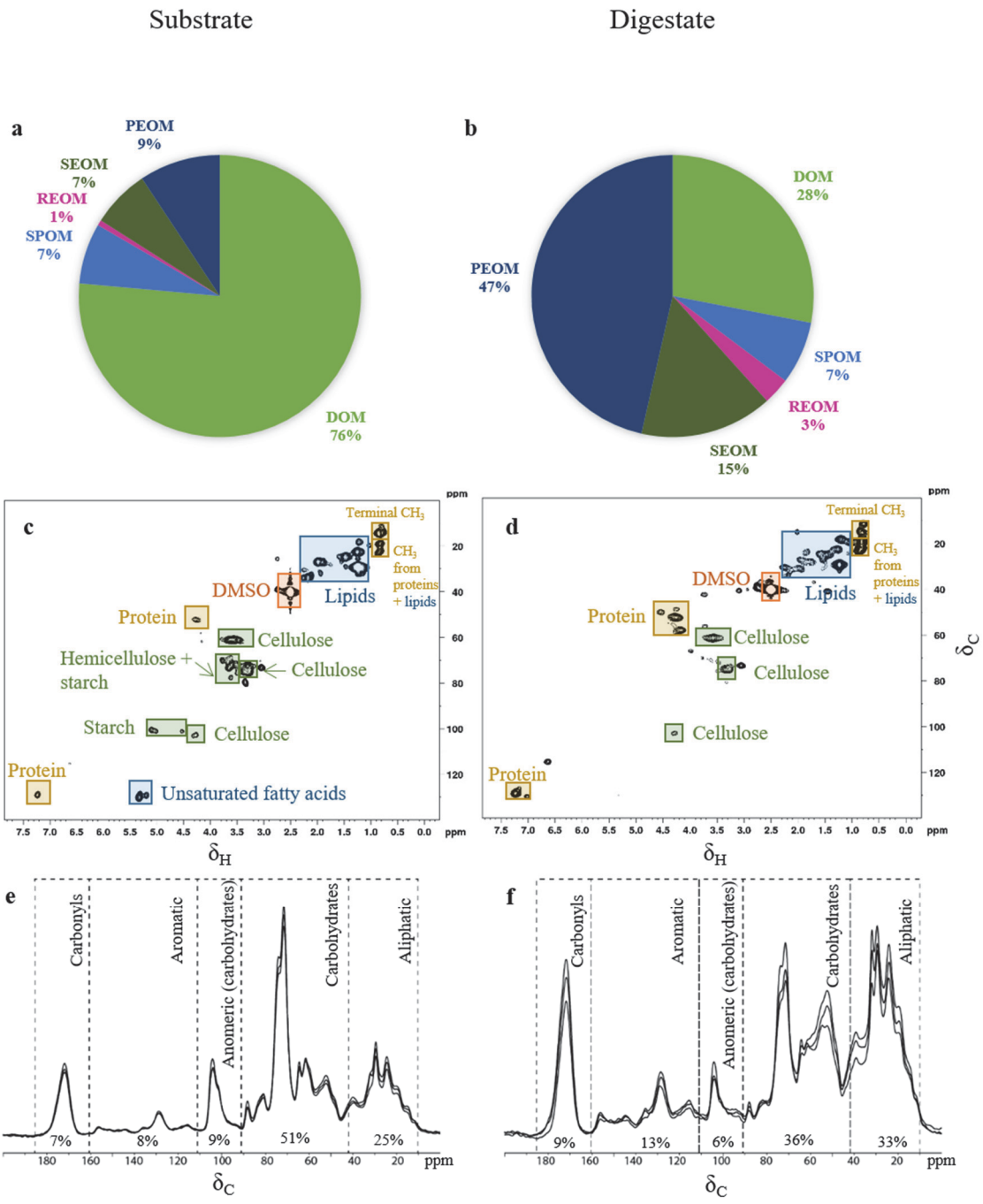


Figure 8. Organic matter characterization of substrate and digestate according to the sequential extraction procedure and NMR spectra. a-b) Relative distribution of organic C extracted after each step of the sequential extraction procedure form substrate and digestate samples. c) 2D ^{1}H-^{13}C HSQC NMR spectrum of DOM solid residue from substrate. d) Corresponding 2D ^{1}H-^{13}C HSQC NMR spectrum from digestate. The x and y-axis reports the chemical shift of ^{1}H and ^{13}C respectively in part per million (ppm) and peak originating from proteins, lipids and carbohydrates are marked in orange, blue and green, respectively. e)^{13}C

CPMAS NMR spectra of DOM solid residues (n:3) from substrate. The integrated peak areas are expressed in % of total integral f) Corresponding spectrum from digestate.

NMR spectroscopy analyses of the solid pellets after each step of the sequential extraction allowed to discern the major structural groups, which remain after application of chemical reagents used for fractionation of particulate organic matter (cf. Table 2).

The solid residues recovered after DOM, SPOM, REOM and SEOM extractions display a comparable distribution of C among different organic groups in both substrate and digestate samples (Figure A1 in Appendix). Only a slight reduction of carbohydrates is observed in the SEOM solid residue in substrate. On the other hand, reduction of C in anomeric and carbohydrate organic groups is found in solid residues of substrate and digestate after PEOM extraction, implying that application of 72% H_2SO_4 resulted in partial dissolution of cellulosic structures.

The ^{1}H-^{13}C HSQC NMR spectra of substrate and digestate solid residues collected after extraction of DOM, SPOM, and REOM fractions are qualitatively similar (Figure A2 and Figure A3). Thus, organic matter extraction by 10 mM $CaCl_2$ and a mixture of 10 mM NaCl and 10 mM NaOH, used for extraction of SPOM and REOM, did not selectively remove organic matter groups from the particulate organic matter. However, the ^{1}H-^{13}C HSQC NMR spectrum of pellet collected after extraction of SEOM fraction from digestate (Figure A3) contains fewer cross peaks within the chemical shift assigned to CH(α) groups of amino acids in peptide chains and proteins ($\delta_{H/C}$: 4.0-4.7/45-62 ppm) (Simpson et al., 2011). Furthermore, several peaks in the aliphatic region where signals from amino acid side-chains appear, *e.g.* the peak at $\delta_{H/C}$: 2.0/15.1 ppm assigned to methionine CH_3-groups (Shakeri Yekta et al., 2018), are absent or have reduced intensities in the spectra of digestate pellet after SEOM extraction. The major peaks in the aromatic region, $\delta_{H/C}$: 6.5-7.4/113-134 ppm, assigned to aromatic side-chains of amino acids (based on comparisons with reference spectra) also experience a reduction in signal intensities after SEOM extraction. Accordingly, the organic matter extracted by 0.1M NaOH in SEOM fraction is related to proteins. It is notable that the presence of protein-derived resonances in the spectra even after SEOM extraction indicates that the proteins were only partially extracted during this step.

Similarly, ^{1}H-^{13}C HSQC NMR spectra in solid residues of substrate (Figure A2) collected after SEOM fraction extraction contain fewer cross peaks from CH(α) groups of the amino acids and from aromatic amino acid side-chains (Simpson et al., 2011). Finally, the ^{1}H,^{13}C HSQC NMR spectra of digestate and substrate solid residue after PEOM extraction (Figure A2 and Figure A3) prove that carbohydrate signals mainly from cellulose, $\delta_{H/C}$: 4.1-5.2/94-106 ppm (anomeric) and $\delta_{H/C}$: 2.9/4.11/59-84 ppm (O-alkyl), and signals

related to amino acids were removed from the solid residue after the PEOM extraction. Thus, the organic matter extracted by 72% H_2SO_4 mainly originate from carbohydrate and partially from proteins available in the samples.

Furthermore, comparison of the amount of C extracted during the sequential extraction procedure and total C content of the samples demonstrates that about 60% of the total initial C was still retained in both the substrate and digestate samples after sequential extraction (Table A6). Nevertheless, the structural characterization of organic matter of NEOM, representing the fraction with low degree bio-accessibility (Jimenez et al., 2015), reveals that it comprises mainly of aliphatic and aromatic CH groups of the protein/biomass fraction of organic matter.

The major organic groups targeted by the chemical reagents used for fractionation of particulate organic matter according to Jimenez et al. (2017, 2015, 2014) and the findings of this study are compared in Table 4. It should be highlighted that in this study NMR spectroscopy was performed on the solid residues recovered at each step of the extraction procedure. In contrast, Jimenez et al. (2017, 2015, 2014) identified the nature of the organic molecules in the liquid fractions extracted by the fractionation procedure. Some similarities are only found in SEOM and PEOM fractions, whereas new organic molecules are found in NEOM fraction, which reflects the nature of substrate analyzed.

Table 4. Target molecules extracted by the sequential extraction procedure adopted by Jimenez et al. (2017, 2015, 2014) and the one performed in this study. The nature of the organic molecules was identified by using reference samples and/or 3D fluorescence spectroscopy in the work of Jimenez et al. (2017, 2015, 2014), whereas NMR spectroscopy was used in this study.

Fractions	Target molecules by Jimenez et al. (2017, 2015, 2014) (Reference samples + 3D fluorescence spectroscopy)	Target molecules according to this study (NMR spectroscopy)
SPOM	Water-soluble proteins and sugars	*The reagent did not selectively remove organic matter*
REOM	Proteins and lipids	*The reagent did not selectively remove organic matter*
SEOM	Humic-like and fulvic like acids, complex proteins (*i.e.* glucolated proteins) and certain lignocellulosic compounds	Certain proteins (CH(α) groups of the amino acids; methionine CH_3-groups; aromatic side-chains of amino acids)
PEOM	Hemicellulose and cellulose	Carbohydrate (hemicellulose, cellulose and starch) and certain proteins

NEOM	Lignin-like compounds and non-extractable humic-like acids (*i.e.* humin)	Protein/biomass (aliphatic and aromatic CH groups)

4.2.1.2 Trace elements fractionation

Concentrations of trace elements extracted at each step during sequential extraction of organic matter are reported in Table 5. Elemental content in CSH fraction is also reported as this fraction may include trace elements likely bound to inorganic ligands.

More than 60% of total Cd, Co, Fe, Mn, Ni and Zn were extracted together with DOM, SPOM, REOM, CSH and SEOM organic fractions of the digestate and substrate samples, whereas it is assumed that the remaining concentrations were in the residual pellet after SEOM extraction. Additionally, sum of As concentrations in the fractions extracted along with sequential extraction of OM from substrate was higher than the As concentrations measured after total digestion of the samples by 7M HNO_3. Molybdenum concentration in the samples is relatively low (2.3±0.1 $\mu g/g_{TSin}$ in digestate, 0.68±0.02 $\mu g/g_{TSin}$ in substrate) and only 13% of total Mo was recovered during the sequential extraction procedure, primarily in DOM and SPOM fraction. The recovery of Cr was also low, *i.e.* 18% and 29% for digestate and substrate, respectively, mainly found in DOM and CSH fractions, whereas only 4% of Cu was recovered in DOM fraction in digestate. In general, the highest concentration of all trace elements is in DOM and CSH fractions of the digestate and substrate. Low concentrations of Al, Fe, Mn, Mo and Ni are found in SPOM fraction of the substrate and digestate, additionally Co and Zn were extracted from substrate in SPOM fraction. Notably, the concentration of five elements is below MLD and MLQ in digestate and substrate. Among the quantified elements, Co, Fe, Mn and Mo were found in REOM fraction of both samples, additionally Al and Ni were extracted from digestate in this fraction. Finally, SEOM contained Al, Cr, Fe, Mn, Ni and Zn with relatively low concentrations extracted from both samples.

Table 5. Trace elements concentration found in each extracted fraction and total elements concentration in digestate (grey rows) and substrate (white rows). Except of DOM fraction extraction, results are mean of triplicate ± standard deviation. Trace elements concentrations in PEOM fraction are omitted due to high degree of uncertainty due to analytical interferences, caused by reagent matrix.

	DOM ($\mu g/g_{TSin}$)	SPOM ($\mu g/g_{TSin}$)	REOM ($\mu g/g_{TSin}$)	CSH ($\mu g/g_{TSin}$)	SEOM ($\mu g/g_{TSin}$)	Total ($\mu g/g_{TSin}$)	% of total content
Al	23.2	1.4 ± 0.1	7.4 ± 0.8	386.6 ± 147.1	44.2 ± 23.6	1359.4 ± 33.7	34%

	7.7	0.9 ± 0.2	<1.0[#]	318.0 ± 8.6	10.3 ± 1.0	722.1 ± 59.7	47%
As	0.6	<0.1*	<0.02[#]	<0.4*	<0.1[#]	1.5 ± 0.1	42%
	0.4	<0.1*	<0.01[#]	<0.2[#]	<0.1[#]	0.22 ± 0.02	180%
Cd	0.01	<0.1[#]	<0.0003[#]	0.13 ± 0.01	<0.004*	0.22 ± 0.01	63%
	0.01	<0.04[#]	<0.01[#]	0.09 ± 0.00	<0.001[#]	0.10 ± 0.00	95%
Co	1.1	<0.2*	0.05 ± 0.00	6.9 ± 0.4	0.28 ± 0.02	10.5 ± 0.1	79%
	2.7	0.35 ± 0.01	0.01 ± 0.00	0.32 ± 0.02	<0.01*	4.04 ± 0.04	83%
Cr	0.3	<0.1[#]	<0.01[#]	0.7 ± 0.1	0.08 ± 0.00	5.9 ± 0.2	18%
	0.2	<0.1[#]	<0.01*	0.32 ± 0.01	0.05 ± 0.00	2.0 ± 0.1	29%
Cu	1.4	<0.9[#]	<0.8[#]	<3.4*	<0.1*	40.4 ± 6.4	4%
	0.2	<0.5[#]	<0.5[#]	6.2±0.1	<0.4[#]	<35.8[§]	-
Fe	471.8	12.8 ± 1.0	9.1 ± 0.7	8502.1 ± 2535.0	68.7 ± 9.0	12623.1 ± 222.8	72%
	749.3	6.8 ± 2.1	2.9 ± 0.3	2119.9 ± 108.3	36.1 ± 1.9	4393.0 ± 71.0	66%
Mn	8.7	1.9 ± 0.1	0.18 ± 0.02	95.0 ± 33.3	0.4 ± 0.1	121.1 ± 5.1	88%
	16.2	4.2 ± 0.1	0.15 ± 0.02	9.0 ± 0.8	0.07 ± 0.00	46.1 ± 1.5	64%
Mo	0.2	0.09 ± 0.01	0.04 ± 0.00	0.01 ± 0.00	<0.1[#]	2.3 ± 0.1	13%

	0.2	0.01 ± 0.00	0.02 ± 0.00	<0.02*	<0.1#	0.68 ± 0.02	28%
Ni	1.6	0.56 ± 0.01	0.17 ± 0.02	17.1 ± 6.2	0.22 ± 0.02	23.5 ± 0.2	84%
	1.3	0.17 ± 0.03	<0.01*	0.5 ± 0.1	0.03 ± 0.01	2.7 ± 0.1	75%
Pb	0.1	<0.1#	<0.04#	1.6 ± 0.3	<0.1*	3.8 ± 0.6	45%
	0.01	<0.05#	<0.02#	0.6 ± 0.1	<0.1*	<1.8§	-
Zn	22.3	<0.7#	<1.1#	109.8 ± 40.3	2.0 ± 0.1	168.4 ± 6.8	80%
	17.2	5.6 ± 0.5	<0.7#	39.3 ± 3.7	1.5 ± 0.3	68.1 ± 0.6	93%

The percentage of total extracted is the ratio between the sum of element's concentration in DOM, SPOM, REOM, CSH and SEOM and the total element' concentration.

*MLQ=average blanks ± 10*standard deviation blanks (n=36) expressed on the same concentration basis (μg/g_{TSin}) as those for the samples using 0.04 L/$g_{TSinitial}$ and 0.02 L/$g_{TSinitial}$ as conversion factor for digestate and substrate respectively.

#MLD=average blanks ± 3*standard deviation blanks (n=36)

§MLQ=average blanks ± 10*standard deviation blanks (n=3), using 0.09 L/$g_{TSinitial}$ as conversion factor.

It should be emphasized that the chemical reagents employed during the sequential extraction procedure may interact with trace elements species in the sample and promote dissolution/precipitation of elements together with organic matter extraction. However, it is not excluded that trace elements extracted together with the operationally defined organic matter fractions, may originate from organically-bound and/or inorganic trace element compounds (*e.g.* CSH fraction) in the samples. To further assess the origin of trace elements in organic matter fractions and assess the contribution of trace element containing minerals during the sequential extraction, an additional sequential extraction procedure was performed where CSH fraction was extracted between DOM and SPOM extraction steps to remove elements bound to minerals. Thus, shifting the extraction of CSH fraction prior to sequential extraction of SPOM, REOM, and SEOM allows the removal of metals bound to minerals, whereas trace elements simultaneously extracted during the subsequent extraction steps represent fractions most likely bound to organic

matter. The reader can refer to Paper I for further details. Results suggested that 31% to 98% and from 61% to 94% of total elemental content, depending on the specific element, are associated with the mineral fraction (*i.e.* CSH fraction) (or strongly bound to organic compounds) in substrate and digestate, respectively, whereas the remaining portion is likely associated with the extracted organic matter fractions. Based on these results and the knowledge of the leaching strength of the reagents used for the sequential extraction procedure, a new distribution of trace elements based on different degree of bio-accessibility is proposed in 4.2.1.3.

4.2.1.3 Implications for simultaneous assessment of bio-accessible trace elements and organic matter

The high concentration of trace elements found in DOM fraction, along with the most bio-accessible organic matter fraction, is related to the presence of dissolved metal species (free ions and complexes with inorganic and organic metal-binding ligands) as well as metal-containing colloids and particles (<0.45 µm). Organic macromolecules such as proteins may contain metals (*e.g.* Co-containing vitamin B12), which contribute to the pool of trace metals associated with DOM (Shakeri Yekta et al., 2014a; Zhu et al., 2014). Therefore, it is hypothesized that trace elements in the DOM fraction are accessible for interaction with the biological interface. Regarding SPOM fraction, obtained by washing the sample pellets with $CaCl_2$ reagent, includes elements that are potentially mobile and bio-accessible since they were released in solution by ion exchange mechanisms with Ca^{2+} or Cl^-. Indeed, $CaCl_2$ reagent is commonly used in soil analysis to extract the exchangeable fraction of trace elements which is also the most available fraction for plant uptake because elements adsorption/desorption processes are strongly related to change in the ionic composition of the sample (Filgueiras et al., 2002; Houba et al., 1996). The trace elements associated with CSH fraction is likely related to trace elements as minerals, such as amorphous metal sulfide, metal carbonate and metal phosphate precipitates, which are dissolved under acidic conditions upon addition of HCl (Albacete et al., 2015; Filgueiras et al., 2002; Rickard and Morse, 2005; Shakeri Yekta et al., 2012). Accordingly, the accessibility of trace elements in the form of inorganic precipitates in solid phase is probably limited for microorganisms. REOM and SEOM fractions include elements extracted from the samples under alkaline condition (pH 11-12). Prevalence of such high pH is uncommon in anaerobic digesters and in environment. Furthermore, dissolution of metal species, which commonly occur at low pH, is unlikely to occur during extraction of REOM and SEOM fraction, implying that trace elements extracted might potentially originate from the simultaneously extracted organic matter. As this fraction of organic matter is considered less accessible, the availability of trace elements associated with REOM and SEOM fractions may as well be limited. Finally, the elements not

extracted by the sequential extraction procedure are very likely not immediately bio-accessible. The degree of bio-accessibility for trace elements in the operationally defined organic matter fractions is presented in Table 6.

Table 6. Degree of bio-accessibility of trace elements in the operationally defined organic matter fractions.

Operationally-defined organic matter fractions	Bio-accessibility degree for trace elements
DOM	High
SPOM	
REOM, CSH, SEOM	
PEOM, non-extracted	Very low

Therefore, a new distribution of trace elements based on different degree of bio-accessibility is proposed Figure 9. In substrate, more than 50% of As, Co, Mn and Ni are bio-accessible or potentially bio-accessible, whereas less than 40% of Fe, Zn and Mo are bio-accessible or potentially bio-accessible. In digestate, except for As, all elements have poor or limited bio-accessibility. In particular, the current results show that less than 20% of Cd, Cr, Cu, Ni, Pb and Zn, considered as harmful elements for plant uptake (Saveyn and Eder, 2014), are immediately bio-accessible or potentially bio-accessible. However, the other elements which serve as nutrient for plants also have poor or no bio-accessibility.

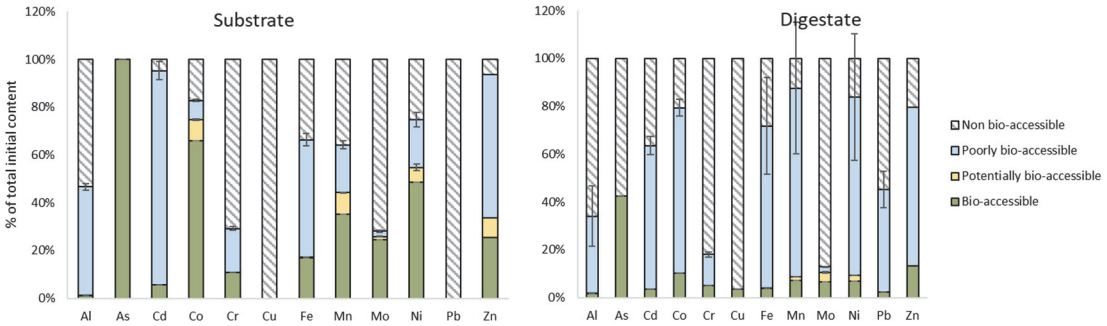


Figure 9.Interpretation of metals fractionation in terms of potential bio accessibility in substrate and digestate.

The new interpretation of trace elements bio-accessibility allows practitioners to perform a single sequential extraction procedure to simultaneously assess trace elements and organic matter bio-accessibility. Knowledge about the C source and micronutrient elements (*i.e.* Co, Fe, Mn, Mo, Ni and Zn) that are immediately and potentially bio-accessible are relevant for the optimal performance of the anaerobic digestion processes. From this, knowledge about the degree of bio-accessibility of harmful elements such as Cd, Cr, Cu, Ni, Pb and Zn (Saveyn and Eder, 2014) for soil microorganisms and plants would be relevant for digestate application as soil amendment.

4.2.2 DGT technique to assess bio-accessible trace elements in digestate: limitations and relevancies

The sequential extraction procedure showed some limitations in trace elements fractionation including a low sensitivity for trace elements extraction in SPOM, REOM and SEOM fractions where 5, 6 and 5 elements out of 12 were not detected or quantified, respectively, in both the substrate and digestate samples. Moreover, it was acknowledged that chemical reagents employed during the sequential extraction procedure may interact with trace elements species in the sample and promote a change in its fractionation. Therefore, it is assumed that DGT technique could improve elemental monitoring in complex matrices as digestate.

Experimental work proved that DGT is a promising technique to assess labile trace elements in digestate samples such as digested sewage sludge (Paper II). Assessing labile fraction of trace elements is of particular interest since it is recognized as the most bio-accessible fraction (Zhang and Davison, 2015). However, results highlighted that the deployment time of DGT samplers, as well as the accumulation of digestate matrix on the diffusive gels should be carefully evaluated to estimate reliable concentrations of labile trace elements (C_{DGT}).

Indeed, results on the optimization of DGT deployment time in the digested sewage sludge showed that the mass of Al, As, Co, Cr (III), Fe, Ni and Mn that accumulated on the binding gel behave, in accordance with theory, linearly over time as showed for Co in Figure 10. The suitable deployment time for the quantified elements is showed in Figure 11, that is up to 18h for As, up to 24h for Mn, from 18 to 48h for Ni, from 4 to 48h for Fe, from 24h to 48h for Cr (III), from 24h to 72h for Al and up to 144h for Co. Therefore, the concentration of DGT-labile elements can be correctly estimated by Eq. (2) using such deployment times. On the other hand, a few elements such as Cd, Cu, Mo were not detected during the experiments (*i.e.* from 4 h to 216 h deployment time). The mass of Pb and Se was below MLD or MLQ up to 24h deployment time and did not accumulate linearly after 24 h (Figure 10). Therefore, the concentration of labile Pb and Se could not be correctly estimated.

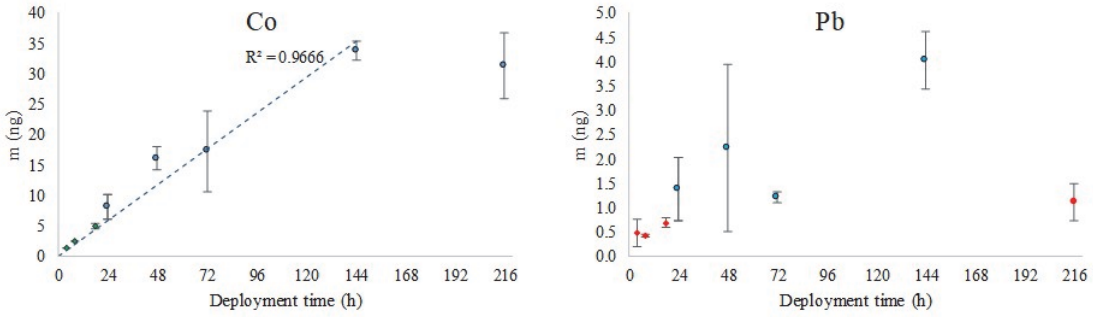


Figure 10. Accumulated mass of Co and Pb versus deployment time during the “short” (green rhombus) and “long term” (blue circles) experiments. In red, values between MLD_{DGT} and MLQ_{DGT}. The 24h point is an average between the two experiments.

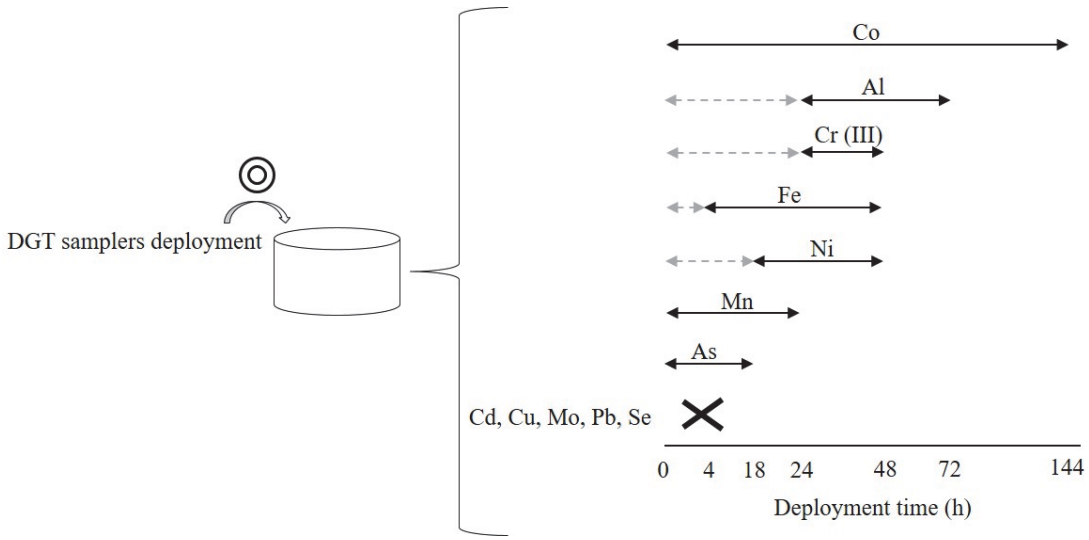


Figure 11. Suitable DGTs deployment times to estimate labile trace elements in the studied digestate sample.

Figure 11 shows that the deployment time varies according to the elements. Such difference is due to deviation from linear accumulation of trace elements on the binding gel. Deviation from linearity can result from saturation of the binding gels and competing effects with other major elements. In fact, when saturation is reached, the accumulated mass of trace elements rapidly decreases because of competing effects between elements. For example, Zr-binding gels, used in this study, are known to bind both As and P (Ding et al., 2010) that are chemical analogous (in the form of arsenate AsO_4^{3-} and phosphate PO_4^{3-}). Consequently, P can replace As on the binding gel. This hypothesis was supported by data shown in Paper II where P displayed the same linear behavior as As, but its accumulated mass on the Zr-binding gel was about 40-fold higher than As up to 24 h deployment time.

Overall, most of the studied elements could be quantified at 24h deployment time in the digested sludge. However, to correctly estimate the concentration of labile trace elements (C_{DGT}) the influence of digestate matrix on trace element accumulation into the DGT devices should considered. It was observed that the matrix of the digested sewage sludge lowers the accumulation of some trace elements in the DGT samplers, probably through interactions of elements with organic matter accumulated in the samplers. Indeed, DGTs pre-exposed to digestate matrix accumulated 11%, 18%, 24%, 28% less Co (II), Ni (II), Pb (II), Cu (II), respectively, compared to the control DGT devices (*i.e.* clean diffusive gel). As (III) and Mo (VI) were below the MLQ of the DGT blanks, whereas no significant ($p>0.05$) under accumulation was observed for Se (IV) and Cd (II) in pre-exposed (*i.e.* “soiled”) DGT samplers. The observed under accumulation of trace elements could be more prominent in real digested sludge compared to the synthetic solution used

in this experimental work (cf. 4.1.4.3) since digestate pH is higher than the one measured in the spiked solution (4<pH<6, Paper II). A high pH is favorable for elements binding to organic matter (Fletcher and Beckett, 1987), at least for cations. Therefore, a correct diffusion rate of trace elements in the presence of digestate matrix should be determined to avoid underestimation of labile trace elements concentrations in digestate.

Additionally, size fractionation of trace elements with restricted and standard diffusive gels showed the presence of large labile complexes for Fe (>1 nm) and small labile complexes for Al, Co, Cr (III) and Mn (<1 nm) in digested sewage sludge (Paper II). This conclusion was reached by comparing the concentration of labile elements accumulated by DGT samplers equipped with restricted or standard diffusive gels (Table 7). Devices with restricted diffusive gels accumulated less Fe ($p<0.01$) compared to standard diffusive gels, whereas the labile concentrations of Al, Co, Cr (III) and Mn accumulated in DGTs with restricted gels was not significantly different from the one accumulated with standard gels ($p>0.05$). A different behavior was observed for As and Ni and labile concentration was higher in DGT samplers equipped with restricted gel than standard gel. Such results are not consistent since restricted gels have smaller pore size (*i.e.* <1 nm) than standard gels (*i.e.* >5 nm), and it should not allow diffusion of a higher amount of labile elements. Such discrepancy could result from the use of a non-adapted diffusion coefficient (D) value for the restricted gels. In fact, the values reported in Table A4 for D in the restricted gel are estimated in synthetic inorganic solutions, whereas it was previously demonstrated that the diffusion of trace elements is affected by the matrix of digestate. Therefore, a proper diffusion coefficient (D) for restricted gels should be estimated in digestate matrix in order to estimate reliable labile elements concentrations.

Table 7. The ratio between C_{DGT} measured in DGT samplers with restricted gel and the DGT samplers with standard gel.

	Al	As	Co	Cr(III)	Fe	Mn	Ni
$C_{labile\ restricted}/C_{labile\ standard}$	0.9	1.3	1.1	1.1	0.7	1.1	1. 3

Finally, it is important to highlight that the DGT technique increases the sensitivity of trace elements monitoring compared to the conventional method of dissolved elements measurement by acid digestion method. Indeed, the MLQ_{DGT} for Al, Cd, Co, Cr (III), Pb and Se was more than 1000 times lower than the $MLQ_{dissolved}$ and for other elements the ratio between $MLQ_{dissolved}$ and MLQ_{DGT} decreases in the following order Fe>Ni>Cu>Mn>As>>Mo. Such high sensitivity of DGT technique is inherent to the sampling method because it concentrates analytes and does not require sample treatment such as liquid-solid separation by centrifugation and dilution, preventing changes in trace

element speciation. Moreover, DGT allowed to quantify 7 out of 12 labile elements, whereas only 3 out of 12 dissolved elements were quantified by dissolved element measurement.

In conclusion, these results highlight the potential of DGT technique to assess labile trace elements in digestate samples. It was observed that 24 h deployment time is a good compromise to quantify most of the studied elements in digested sewage sludge. However, digestate matrix decreased the accumulation of labile elements in DGT devices, and therefore further studies are needed to estimate a correct coefficient of diffusion in digestate sample. It is however recommended to limit interpretation of labile concentration to general trends such as evolution of labile trace elements over time, in order to limit misinterpretation of the absolute DGT labile trace elements concentrations.

4.2.3 Changes in trace elements bio-accessibility in digestate exposed to air

After validation of DGT method to fractionate trace elements in digestate matrix (Paper II), the effect of atmospheric air on the mobility and bio-accessibility of trace elements (*i.e.* labile, soluble and particulate fractions) in digestate was investigated (Paper III). The exposure of digestate to air promoted a change in distribution of DGT-labile, soluble and particulate trace elements. It was assumed that the observed changes in trace element distribution could help to anticipate phenomena related to air exposure occurring in field during digestate management such as digestate storage in open tanks or handling before spreading on land.

Results of this study, performed at laboratory-scale under controlled conditions, showed that soluble Al, As, Co, Cr, Cu, Fe, Mn, Mo and Pb increased during 89 days of digestate aeration (Figure A4). In particular, a high increase of soluble Al, Co, Cr, Cu, Fe, Mn and Mo was observed from day 79 when digestate aeration was enhanced. As a typical example the trend of soluble Fe is displayed in Figure 12. The concentration of soluble As was quite constant during forced aeration as shown in Figure 12. Trace elements release in solution was likely caused by direct oxidation of sulfur precipitates in presence of oxygen from the air (Fermoso et al., 2015). Sulfide oxidation leads to metal sulfide precipitates dissolution (*e.g.* FeS, CoS, CuS, PbS (Legros et al., 2017; Maharaj et al., 2018; Möller and Müller, 2012)) as well as the release of sulfate. Indeed, a significant increase of sulfate concentration was measured after the 57^{th} days of passive aeration and during forced aeration as shown in Figure 13. It should be also mentioned that soluble Cd, Ni, Sb, Se and W were below the method's detection limit or quantification (*i.e.* lower than 12, 721, 102, 1077 and 69 µg/L, respectively) during the whole experiment, and therefore cannot be discussed.

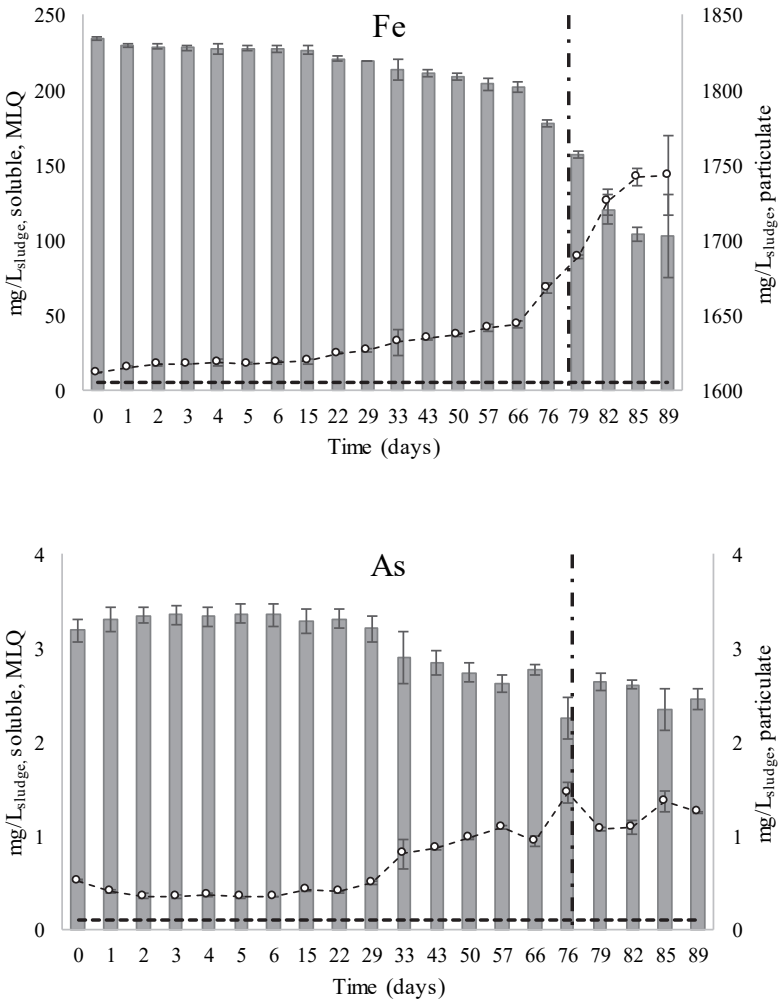


Figure 12. Examples of soluble (dashed line with circles) and particulate (bars) elements' concentration over time. The bold horizontal dashed line is the method limit of quantification (MLQ) for soluble elements whereas the vertical dashed line indicates the beginning of forced aeration.

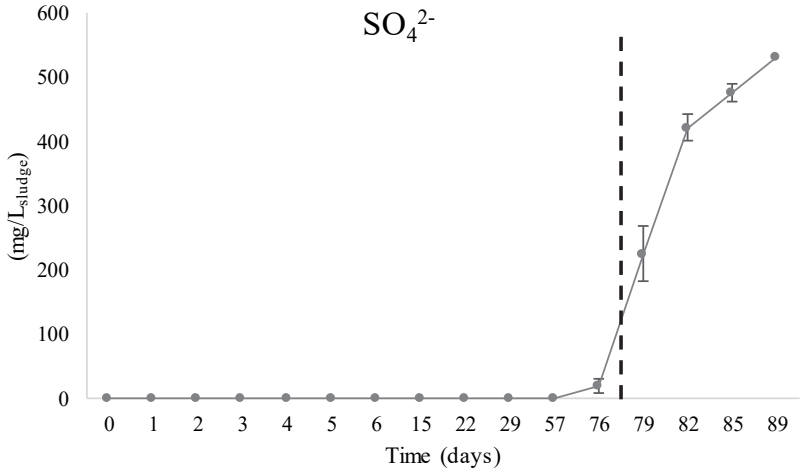
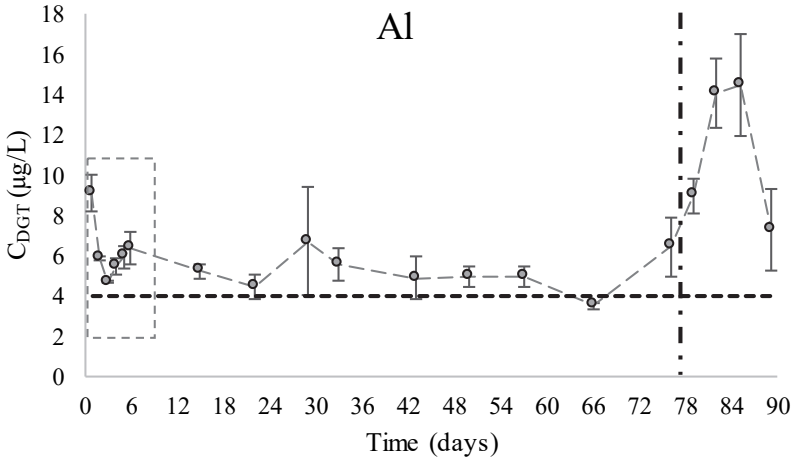


Figure 13. Sulfate measured over time. The vertical dashed line indicates the beginning of forced aeration.

Labile Al, Mo, Ni, Sb, Se and W increased only during forced aeration (Figure A5 and Figure 14), whereas their concentration was rather constant or close to the MLD_{DGT} during passive aeration (*i.e.* up to day 76). However, the observed rapid increase for those labile concentration was followed by a decrease, except for Mo and W as shown in Figure A5 and Figure 14. Arsenic and Co slightly decreased immediately after forced aeration and their concentration increased again at the 85th day. Different from other elements, labile Fe and Mn concentrations continued to decrease over time, even under forced aeration. Whereas, labile Cd, Cr(III), Cu and Pb concentrations were not detected or quantified during the whole experiment.



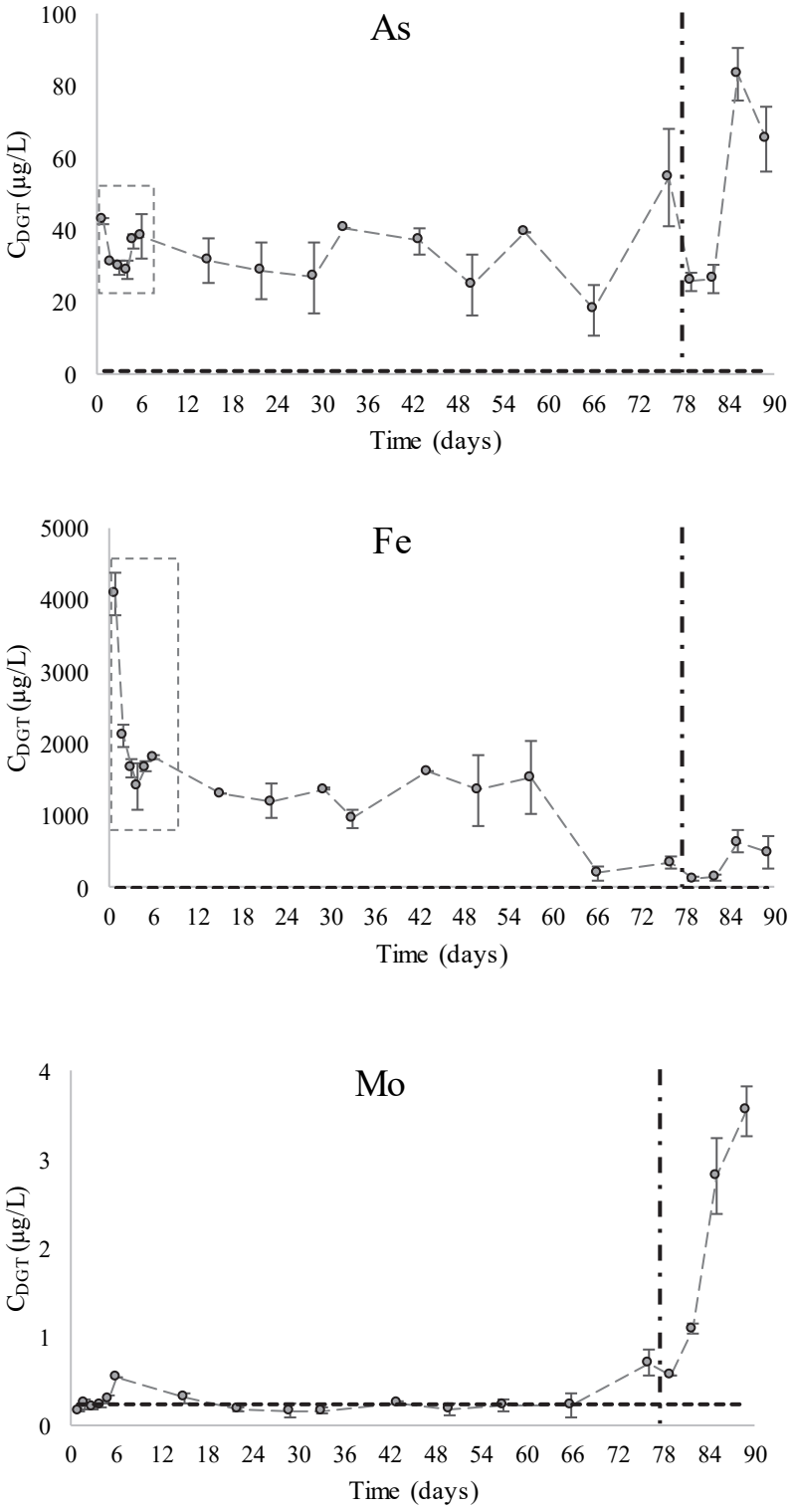


Figure 14. Examples of labile elements' concentration over time. The bold horizontal dashed line is the MLQ_{DGT} whereas the vertical dashed line indicates the beginning of forced aeration.

The increase of labile elements during forced aeration could be a direct consequence of their release form sulfide species as previously discussed. In contrast, the decrease of Fe and Mn labile concentration was not associated with the increase of their soluble fraction, especially at the end of the forced aeration, meaning that part of these soluble elements were DGT-inert (*e.g.* colloids such as Fe(II)-phosphate or strongly complexed with organic functional groups such as thiol groups (Shakeri Yekta et al., 2014b)). Therefore, it is assumed that oxidation converted a part of labile species of Fe and Mn into soluble non-labile species. Conversely, the delay observed for the increase of labile As and Co concentration during forced aeration allowed slow conversion into labile forms. Moreover, adsorption onto Fe/Mn colloids could have also occurred.

In conclusion, passive and forced aeration resulted both in a release in solution of Al, As, Co, Cr, Cu, Fe, Mn, Mo and Pb, which was certainly due to oxidation of metal sulfide precipitates. However, under passive aeration, dissolution was slow during the first four weeks, whereas under forced aeration the dissolution of all the quantified elements was high, except for As. It was assumed that the observed increase of trace elements mobility due to aeration may likely occur during storage in open tanks or digestate handling before application on land. This dissolution did not promote an increase of DGT-labile concentrations during passive aeration. Conversely, forced aeration promoted an increase of the labile Al, As, Co, Mo, Ni, Sb, Se and W. Therefore, it can be assumed that passive aeration of digestate like in open storage tanks would not increase trace elements bioaccessibility unless significant aeration such as during digestate handling for land spreading takes place.

5 Conclusions and Future Outlook

Application of an organic matter fractionation procedure was used to simultaneously assess organic matter and trace element bio-accessibility in substrate and digestate (Paper I). It was demonstrated that more than 60% of total As, Cd, Co, Fe, Mn, Ni and Zn were extracted, whereas the extraction of Al, Cr, Cu, Mo and Pb was limited. It was observed that trace elements were mainly recovered in DOM and CSH fractions, which were defined as the immediately and poorly bio-accessible fractions, respectively. It was proved that 31% to 98% and from 61% to 94% of total elemental content, depending on the specific element, were most likely associated with minerals (CSH fraction) in both substrate and digestate, whereas the remaining elements were associated with organic matter. However, further studies could be implemented to assess the speciation of trace-elements in the fractions having a high degree of mobility according to the organic matter fractionation procedure (*i.e.* DOM and SPOM fractions). NMR spectroscopy analyses allowed to identify proteins in SEOM fraction, while carbohydrate and certain proteins were identified in PEOM fraction. Overall, the sensitivity of the extraction procedure was questioned since 5 to 6 elements out of 12 were not detected or quantified in certain organic matter fractions. Moreover, the chemical reagents employed during the extraction procedure likely promoted a dissolution/precipitation of trace elements, therefore a change in their fractionation biasing interpretation of the procedure.

Subsequently, the prediction of bio-accessible trace elements, more specifically labile trace elements, was achieved by DGT fractionation technique (Paper II). Firstly, it was demonstrated that DGT technique can assess labile trace elements in digested sewage sludge. However, DGT deployment time should be carefully evaluated in digestate. It was observed that short deployment (*i.e.* lower that 4 h) led to insufficient element accumulation or non-establishment of steady state in DGT samplers, while long deployment (from 18 to 144 h depending on the element) led to saturation of the binding gels and/or

competing effects with other major elements. Moreover, it was proved that digestate matrix lowers trace elements accumulation from 10 to 30%, depending on the element, leading to underestimation of DGT labile concentrations. Therefore, further studies are needed to estimate the diffusion coefficient of trace elements for standard gels in digestate samples to accurately estimate labile element concentrations. Such coefficient of diffusion could be determined on “soiled” diffusive gels (cf. section 4.1.4.3) using the diffusion cell method (Scally et al., 2006; Zhang and Davison, 1995). However, it was observed that DGT technique greatly increased the sensitivity for elemental monitoring than measurement of dissolved elements. Given that DGT technique is more sensitive than conventional measurements of dissolved elements, it was possible to assess the general trend of labile trace elements concentration over time in digestate exposed to air (Paper III). It was observed that aeration promoted dissolution of Al, As, Co, Cr, Cu, Fe, Mn, Mo and Pb, which was certainly due to oxidation of metal sulfide precipitates. Therefore, it was assumed that the observed increase of trace element mobility due to aeration may likely occur during storage in open tanks or digestate handling before application on land. However, this dissolution did not promote an increase of DGT-labile concentrations during passive aeration. Conversely, forced aeration promoted an increase of the labile Al, As, Co, Mo, Ni, Sb, Se and W. Therefore, it was assumed that an increase of labile trace elements would occur when significant aeration takes place such as during digestate handling for spreading on land. It was also suggested that the bio-accessibility of labile elements could increase after land application depending on the soil’s sorption capacity and plants uptake mechanisms. Such hypothesis should be further explored for risk assessment, *e.g.* DGT samplers could be deployed in soils amended with digestate and the mobility of labile and soluble elements could be monitored over time. Additionally, the change in speciation of sulfur compounds in the liquid and solid phases of digestate could be investigated during exposure of digestate to atmospheric air.

In summary, the advantages and limitations of two fractionation techniques were highlighted in this study. Overall, the techniques are valuable to estimate bio-accessible trace elements for environmental risk assessments of digestate utilization as a soil amendment. Therefore, fractionation techniques should be preferred to total elements content measurements in order to provide information on the mobility, accessibility and potential bioavailability of trace elements in digestates. However, further studies are needed to estimate the correct coefficient of diffusion of trace elements in digestates to assess DGT labile concentrations using the standard and restricted diffusive gels. Moreover, DGT devices could be employed to assess labile elements in the DOM and SPOM liquid fractions extracted by the organic matter sequential extraction procedure, since they were defined the most bio-accessible fractions of the organic matter and trace elements.

Therefore, additional information regarding the accessibility of trace elements in substrate and digestate could be provided. To further address bio-availability, additional tests are needed to correlate results obtained from fractionation techniques (*e.g.* sequential extraction procedures, DGT technique) with biological measurements using plants exposed soils amended with digestate. Such findings could help scientists and policy makers to define new threshold values for trace element concentrations in digestate, taking into account the bio-accessible and bio-available fractions.

Appendix

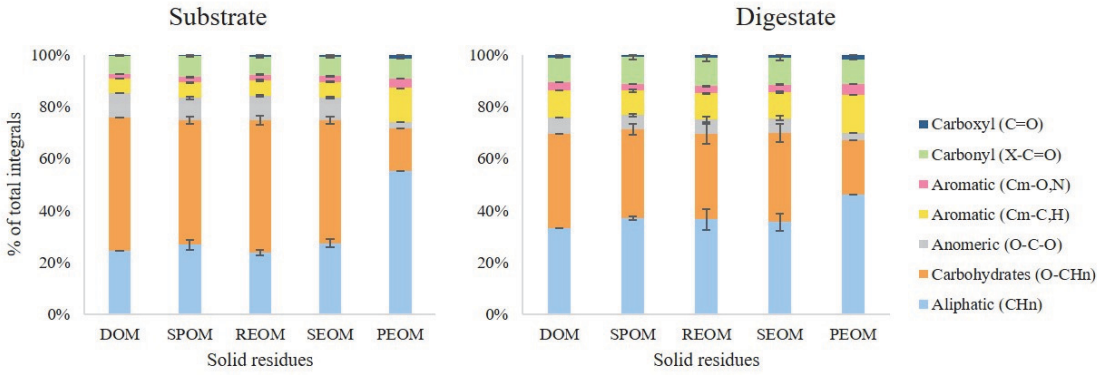


Figure A1. Distribution of C among different organic groups for the substrate and digestate solid residues. Results are expressed as % of total integrals of ^{13}C CPMAS NMR spectra (Paper I).

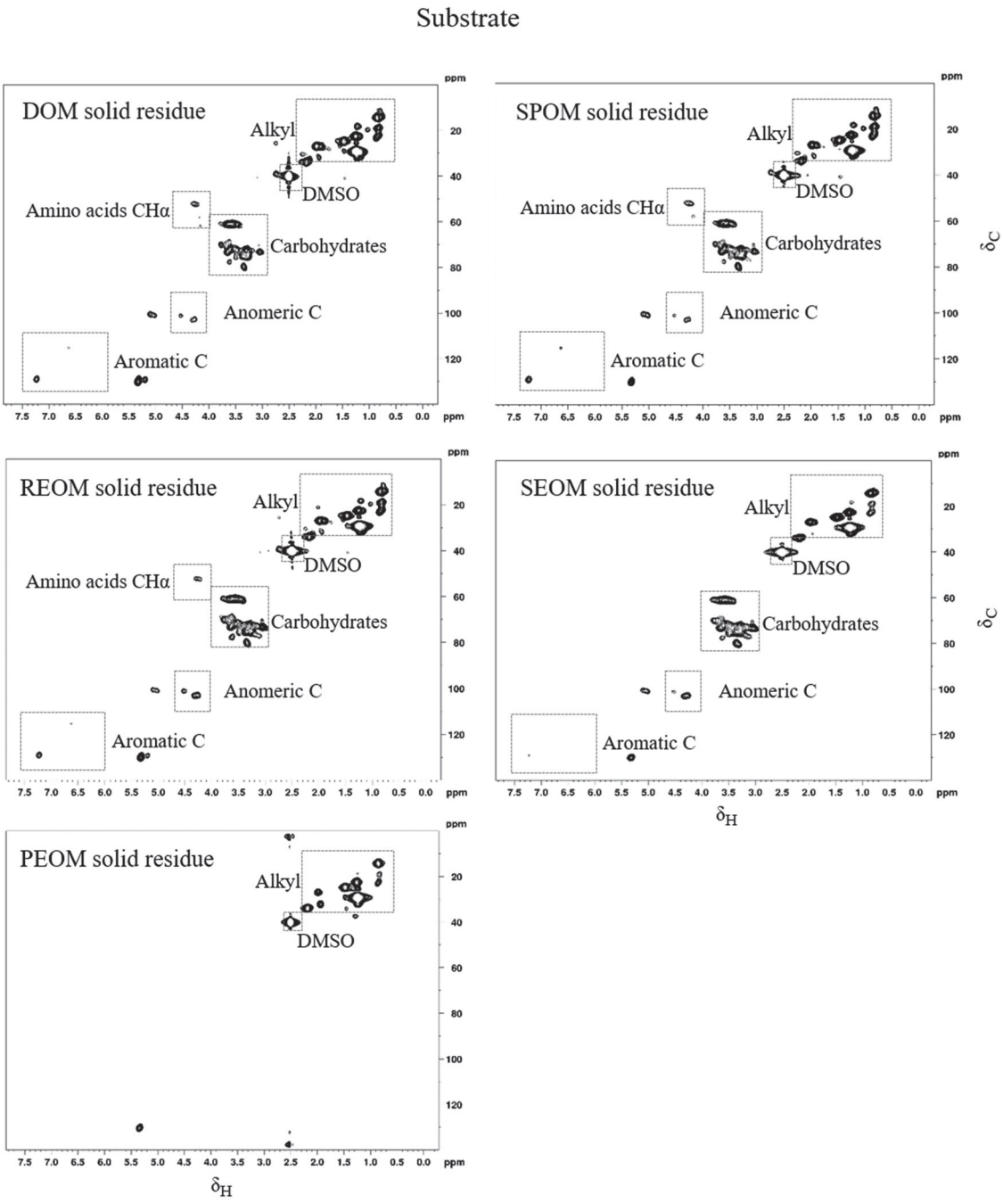


Figure A2. 2D ^{1}H-^{13}C HSQC NMR spectra of substrate solid residues collected during the sequential extraction procedure. Spectral regions representative for different chemical structures or functionalities are indicated by rectangles (Simpson et al., 2011; Soucémarianadin et al., 2017) (Paper I).

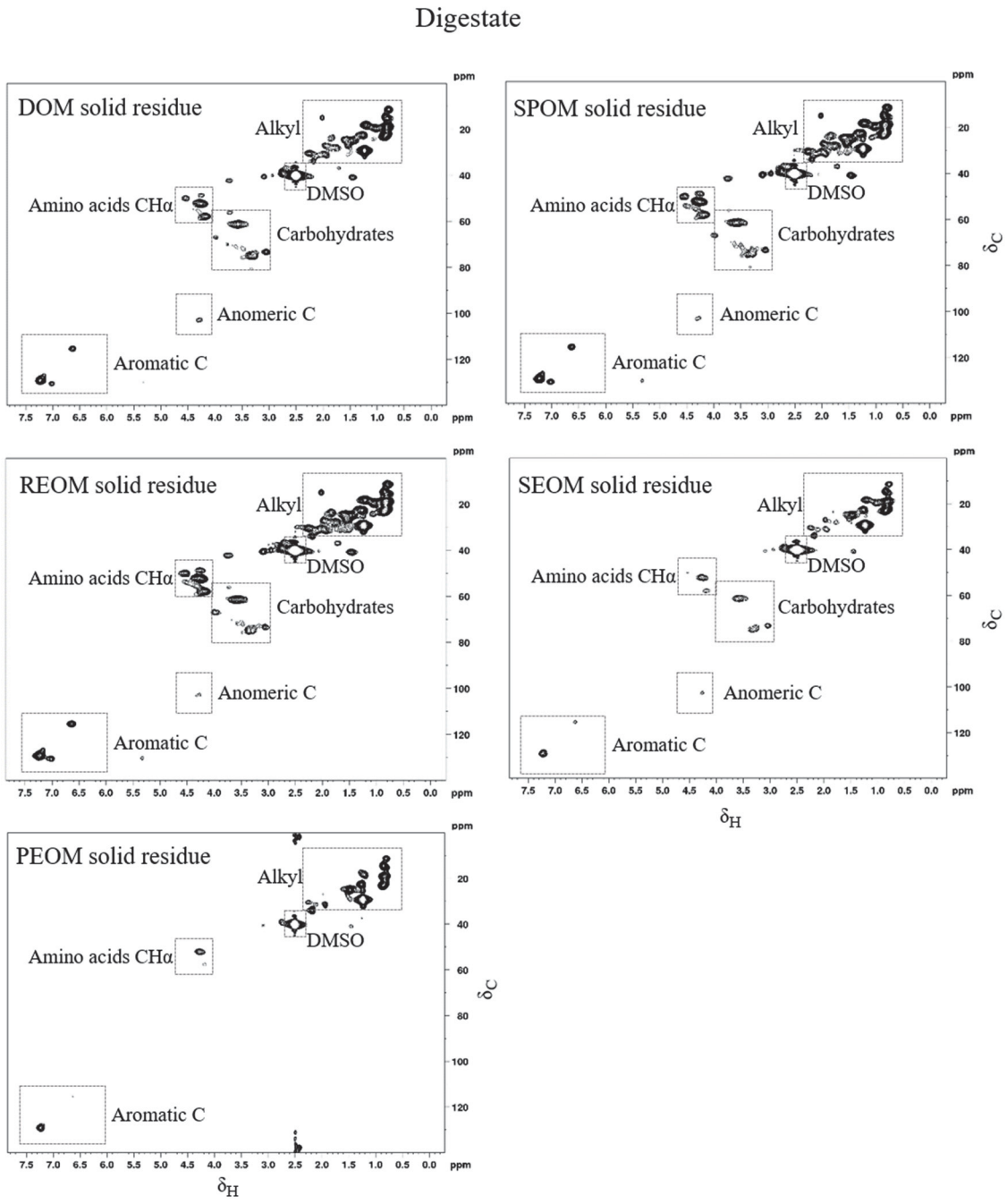
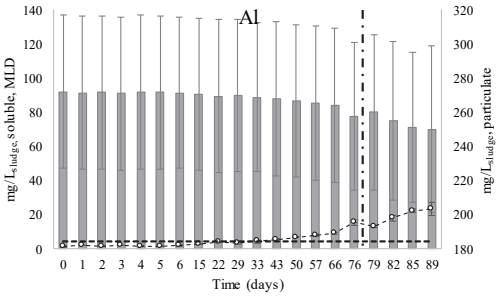
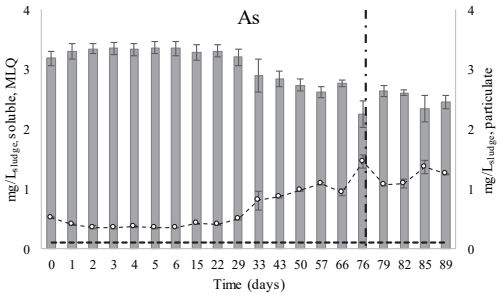
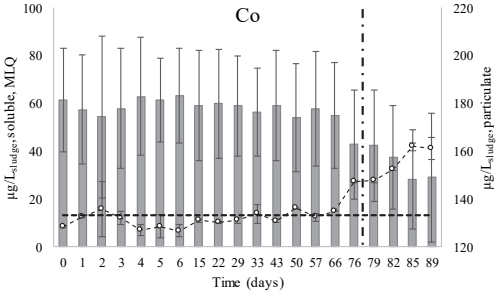
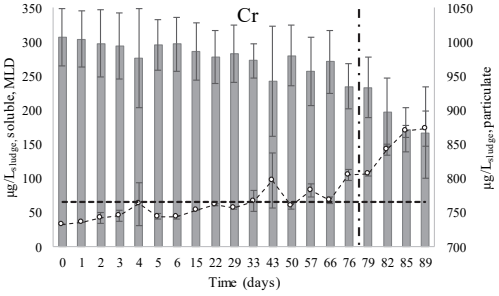


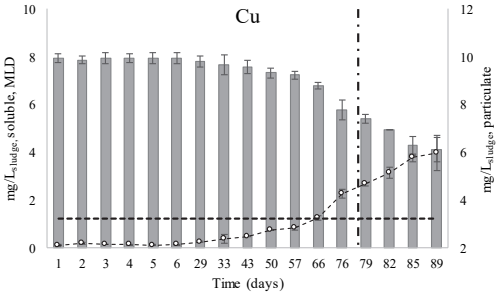
Figure A3. 2D ^{1}H-^{13}C HSQC NMR spectra of digestate solid residues collected during the sequential extraction procedure (Paper I).

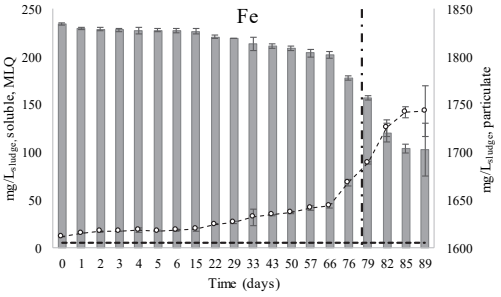

Al
mg/L sludge, soluble, MLD
mg/L sludge, particulate
Time (days)

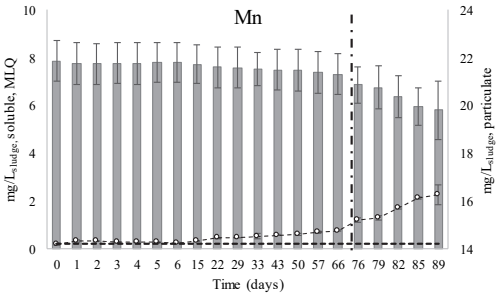

As
mg/L sludge, soluble, MLQ
mg/L sludge, particulate
Time (days)

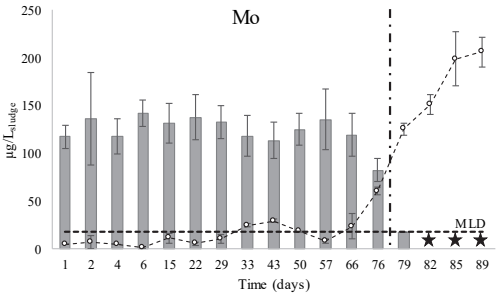

Co
µg/L sludge, soluble, MLQ
µg/L sludge, particulate
Time (days)


Cr
µg/L sludge, soluble, MLD
µg/L sludge, particulate
Time (days)


Cu
mg/L sludge, soluble, MLD
mg/L sludge, particulate
Time (days)


Fe
mg/L sludge, soluble, MLQ
mg/L sludge, particulate
Time (days)


Mn
mg/L sludge, soluble, MLQ
mg/L sludge, particulate
Time (days)


Mo
µg/L sludge
MLD
Time (days)

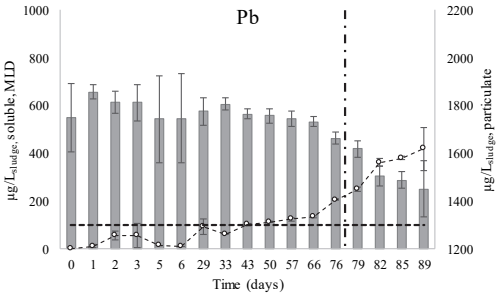
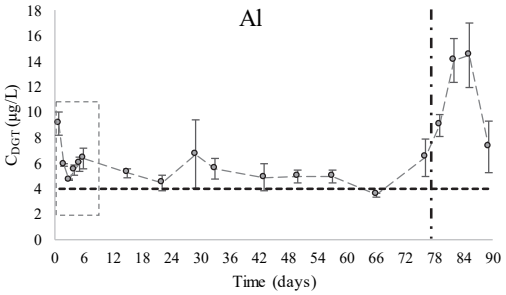
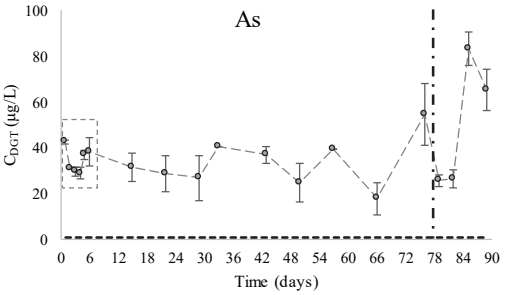
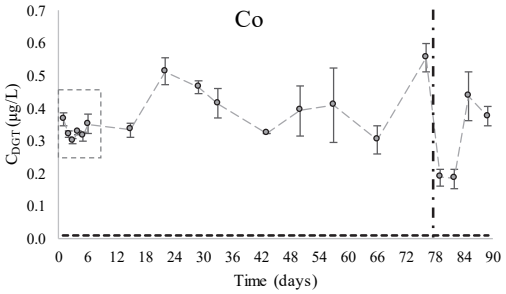
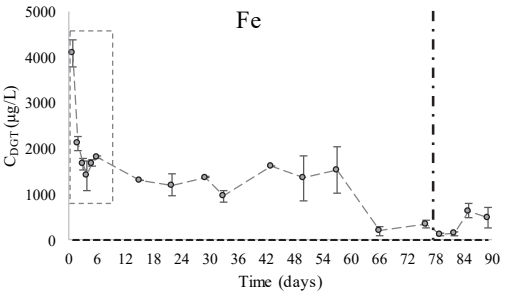

Pb
µg/L sludge, soluble, MLD
µg/L sludge, particulate
Time (days)

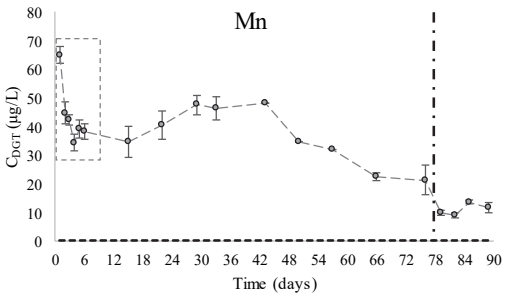
Figure A4. Soluble (dashed line with circles) and particulate (bars) elements' concentration over time. The bold horizontal dashed line is the method limit of detection (MLD) or quantification (MLQ) for soluble elements whereas the vertical dashed line indicates the beginning of forced aeration. When the soluble prevails the particulate fraction a black star replaces the bar (Paper III).

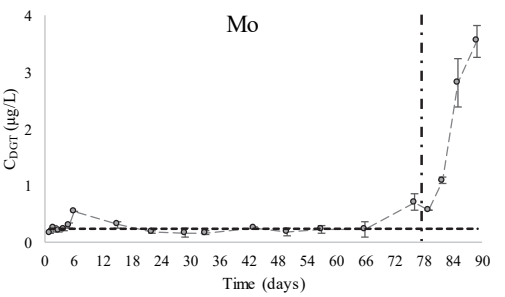

Al
C_{DGT} (µg/L)
Time (days)

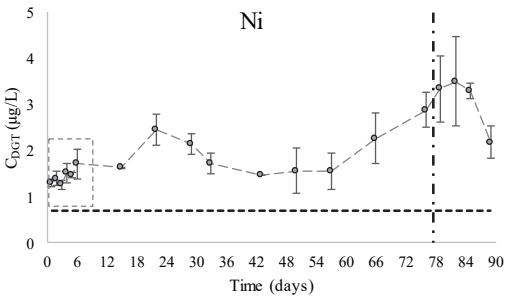

As
C_{DGT} (µg/L)
Time (days)

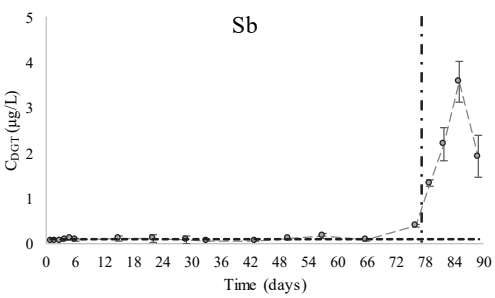

Co
C_{DGT} (µg/L)
Time (days)


Fe
C_{DGT} (µg/L)
Time (days)


Mn
C_{DGT} (µg/L)
Time (days)


Mo
C_{DGT} (µg/L)
Time (days)


Ni
C_{DGT} (µg/L)
Time (days)


Sb
C_{DGT} (µg/L)
Time (days)

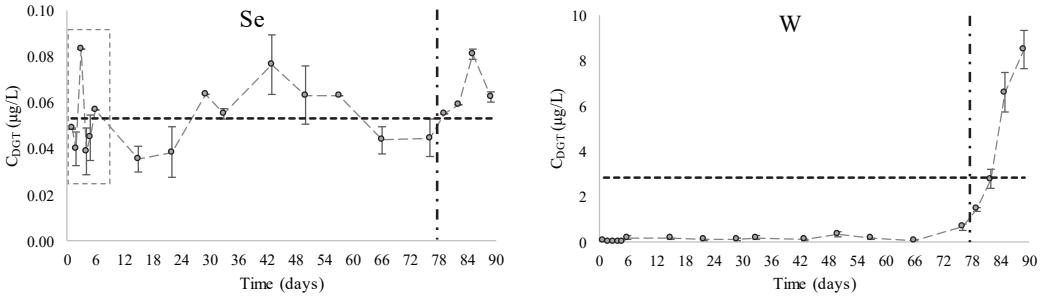


Figure A5. Labile trace elements (grey dashed line with grey circles) monitored over time in digested sewage sludge. The bold horizontal dashed line is the MLD_{DGT} or MLQ_{DGT} whereas the vertical dashed line indicates the beginning of forced aeration (Paper III).

Table A1. Parameters measured at the beginning and at the end of the experiment (Paper II). Concentration of the elements added in the two solutions to assess the interference of digestate matrix on trace elements accumulation.

	Measured parameters		Added element (µg/L)	
Solution of 10^{-2} M NaCl + cations	pH_{start}	4.2	Co(II)	71
	pH_{end}	4.4	Ni(II)	128
	T_{start}	19 °C	Pb(II)	72
	T_{end}	22 °C	Cu(II)	30
			Cd(II)	67
Solution of 10^{-2} M NaCl + anions	pH_{start}	5.5	As(III)	22
	pH_{end}	6.1	Se(IV)	7
	T_{start}	19 °C	Mo(VI)	7
	T_{end}	22 °C		

Table A2. Elution factors fe from the literature with elution conditions similar to the work in Papers II and III.

	Elution factor	Reference
Al	0.85	(Devillers et al., 2017)
As	0.70	Result not published
Cd	0.85	(Devillers et al., 2017)
Co	0.85	(Devillers et al., 2017)
Cr	0.80	(Devillers et al., 2017)
Cu	0.85	(Devillers et al., 2017)
Fe	0.70	(Zhang and Davison, 1995)
Mn	0.82	(Zhang and Davison, 1995)
Mo	0.86	Result not published
Ni	0.85	(Devillers et al., 2017)
P	0.95	(Ding et al., 2010)
Pb	0.85	(Devillers et al., 2017)
Sb	0.61	Result not published
Se	0.86	Result not published
W	0.70	Result not published

Table A3. Coefficients of diffusion in a standard diffusive gel taken from the literature and used in Papers II and III. All values are referred to 25°C.

	$D_{standard}$ (cm^2/sec)
Al	$4.75 \cdot 10^{-6}$[a]
As	$6.90 \cdot 10^{-6}$[b] (Paper III); $8.29 \cdot 10^{-6}$[c] (Paper II)
Cd	$6.09 \cdot 10^{-6}$[a]
Co	$5.94 \cdot 10^{-6}$[a]
Cr (III)	$5.05 \cdot 10^{-6}$[a]
Cu	$6.23 \cdot 10^{-6}$[a]
Fe	$6.11 \cdot 10^{-6}$[a]
Mn	$5.85 \cdot 10^{-6}$[a]
Mo	$6.62 \cdot 10^{-6}$[b]
Ni	$5.77 \cdot 10^{-6}$[a]
P	$6.05 \cdot 10^{-6}$[a]
Pb	$8.03 \cdot 10^{-6}$[a]
Sb	$6.92 \cdot 10^{-6}$[b]
Se	$7.77 \cdot 10^{-6}$[b] (Paper III); $7.07 \cdot 10^{-6}$[c] (Paper II)
W	$6.05 \cdot 10^{-6}$[b]

[a] Reference: http://www.dgtresearch.com/diffusion-coefficients/

[b] Reference: (Wang et al., 2016)

[c] Reference: (Bennett et al., 2010)

Table A4. The ratio of the restricted diffusion coefficients to the standard diffusion coefficients estimated by experimental works in different laboratories. These values were used only in Paper II.

Element	$D_{restricted}/D_{standard}$
Al	0.72[b]; 0.68[c]
As	0.71[b]; 0.71[c]
Cd	0.62[a]; 0.73[b]; 0.72[c]
Co	0.76 [b]; 0.71[c]
Cr	-
Cu	0.70[a]; 0.78[b]; 0.72[c]
Fe	-
Mn	0.78[b]; 0.71[c]
Mo	0.68[b]; 0.71[c]
Ni	0.69[a]; 0.72[b]; 0.72[c]
Pb	0.72[a]; 0.72[b]; 0.73[c]
Se	-

[a] Estimated by the diffusion cell method. Reference (Scally et al., 2006)

[b] Estimated by the DGT time-series method at pH 4. Reference (Shiva et al., 2015)

[c] Estimated by the diffusion cell method at pH 4. Reference (Shiva et al., 2015)

Table A5. Acquisition parameters for NMR spectroscopy analysis (Paper I).

NMR analyses	Required amount of sample	Operating frequency	Pulse sequence	Number of scans	Additional acquisition parameters
^{13}C CPMAS	90 ± 10 mg	125.75 MHz	cp	3500	Relaxation delay 1 s Contact-time 1 ms Spin-rate 10 kHz Total exp. time 1 h
^{1}H	20 ± 2 mg	600 MHz	zg30	8	Relaxation delay 1.5 s Total exp. time 30 s
^{1}H-^{13}C HSQC	20 ± 2 mg	600 MHz	hsqcetgpsisp2.2	16	Relaxation delay 2 s t1-increments 128 Total exp. time 70 min

Table A6.Mass balance of C. Extracted dissolved organic C and initial C content are compared to estimate the percentage of non- and extracted C (Paper I).

	Substrate	Digestate
Extracted dissolved organic C* (mg)	213.6	121.4
Initial C content[#] (mg)	546.0	316.9
Extracted C (%)	39.1	38.3
Non-extracted C (%)	60.9	61.7

*It is the sum of dissolved organic C extracted in SPOM, REOM, SEOM and PEOM fractions

[#]It is quantified in the solid residue after DOM extraction

References

Albacete, M.G., Tarquis, A.M., Cartagena, M.C., 2015. Risk of Leaching in Soils Amended by Compost and Digestate from Municipal Solid Waste 2014, 1–12. https://doi.org/10.1155/2014/565174

Bennett, W.W., Teasdale, P.R., Panther, J.G., Welsh, D.T., Jolley, D.F., 2010. New diffusive gradients in thin films technique for measuring inorganic arsenic and selenium (IV) using a titanium dioxide based adsorbent. Anal. Chem. 82, 7401–7407.

Bonetta, S., Bonetta, S., Ferretti, E., Fezia, G., Gilli, G., Carraro, E., 2014. Agricultural reuse of the digestate from anaerobic co-digestion of organic waste: Microbiological contamination, metal hazards and fertilizing performance. Water. Air. Soil Pollut. 225, 1–11. https://doi.org/10.1007/s11270-014-2046-2

Bourven, I., Casellas, M., Buzier, R., Lesieur, J., Lenain, J.F., Faix, A., Bressolier, P., Maftah, C., Guibaud, G., 2017. Potential of DGT in a new fractionation approach for studying trace metal element impact on anaerobic digestion: the example of cadmium. Int. Biodeterior. Biodegrad. 119, 188–195. https://doi.org/10.1016/j.ibiod.2016.11.007

Braga, A.F.M., Zaiat, M., Silva, G.H.R., Fermoso, F.G., 2017. Metal fractionation in sludge from sewage UASB treatment. J. Environ. Manage. 193, 98–107. https://doi.org/10.1016/j.jenvman.2017.01.070

Callander, I.J., Barford, J.P., 1983. Precipitation, chelation, and the availability of metals as nutrients in anaerobic digestion. I. Methodology. Biotechnol. Bioeng. 25, 1947–1957. https://doi.org/10.1002/bit.260250805

Cestonaro do Amaral, A., Kunz, A., Radis Steinmetz, R.L., Justi, K.C., 2014. Zinc and copper distribution in swine wastewater treated by anaerobic digestion. J. Environ. Manage. 141, 132–137. https://doi.org/10.1016/j.jenvman.2014.03.021

D'Abzac, P., Bordas, F., Joussein, E., van Hullebusch, E., Lens, P.N.L., Guibaud, G., 2013. Metal binding properties of extracellular polymeric substances extracted from anaerobic granular sludges. Environ. Sci. Pollut. Res. 20, 4509–4519. https://doi.org/10.1007/s11356-012-1401-3

D'Abzac, P., Bordas, F., van Hullebusch, E., Lens, P.N.L., Guibaud, G., 2010. Effects of extraction procedures on metal binding properties of extracellular polymeric substances (EPS) from anaerobic granular sludges. Colloids Surfaces B Biointerfaces 80, 161–168. https://doi.org/10.1016/j.colsurfb.2010.05.043

Devillers, D., Buzier, R., Charriau, A., Guibaud, G., 2017. Improving elution strategies for Chelex®-DGT passive samplers. Anal. Bioanal. Chem. 409, 7183–7189. https://doi.org/10.1007/s00216-017-0680-4

Devillers, D., Buzier, R., Simon, S., Charriau, A., Guibaud, G., 2016. Simultaneous measurement of Cr(III) and Cr(VI) in freshwaters with a single Diffusive Gradients

in Thin Films device. Talanta 154, 533–538. https://doi.org/10.1016/j.talanta.2016.04.009

Ding, S., Xu, D., Sun, Q., Yin, H., Zhang, C., 2010. Measurement of dissolved reactive phosphorus using the diffusive gradients in thin films technique with a high-capacity binding phase. Environ. Sci. Technol. 44, 8169–8174. https://doi.org/10.1021/es1020873

Ernstberger, H., Zhang, H., Davison, W., 2002. Determination of chromium speciation in natural systems using DGT. Anal. Bioanal. Chem. 373, 873–879. https://doi.org/10.1007/s00216-002-1370-3

European Commission, 2018a. Establishing best available techniques (BAT) conclusions for waste treatment, under Directive 2010/75/EU of the European Parliament and of the Council. Off. J. Eur. Union 38–90.

European Commission, 2018b. A sustainable Bioeconomy for Europe: strengthening the connection between economy, society and the environment. https://doi.org/10.2777/478385

European Commission, 1986. Directive 86/278/EEC Protection of the Environment, and in particular of the soil, when sewage sludge is used in agriculture. Off. J. Eur. Communities 4, 6–12.

Feng, X.M., Karlsson, A., Svensson, B.H., Bertilsson, S., 2010. Impact of trace element addition on biogas production from food industrial waste--linking process to microbial communities. FEMS Microbiol. Ecol. 74, 226–40. https://doi.org/10.1111/j.1574-6941.2010.00932.x

Fermoso, F.G., van Hullebusch, E.D., Guibaud, G., Collins, G., Svensson, B.H., Carliell-Marquet, C., Vink, J.P.M., Esposito, G., Frunzo, L., 2015. Fate of Trace Metals in Anaerobic Digestion, in: Guebitz, G.M., Bauer, A., Bochmann, G., Gronauer, A., Weiss, S. (Eds.), Biogas Science and Technology. Springer International Publishing, Switzerland, pp. 171–195. https://doi.org/10.1007/978-3-319-21993-6_7

Filgueiras, A. V., Lavilla, I., Bendicho, C., 2002. Chemical sequential extraction for metal partitioning in environmental solid samples. J. Environ. Monit. 4, 823–857. https://doi.org/10.1039/b207574c

Fletcher, P., Beckett, P.H.T., 1987. The chemistry of heavy metals in digested sewage sludge-II. Heavy metal complexation with soluble organic matter. Water Res. 21, 1163–1172. https://doi.org/10.1016/0043-1354(87)90167-9

Formentini, T.A., Legros, S., Fernandes, C.V.S., Pinheiro, A., Le Bars, M., Levard, C., Mallmann, F.J.K., da Veiga, M., Doelsch, E., 2017. Radical change of Zn speciation in pig slurry amended soil: Key role of nano-sized sulfide particles. Environ. Pollut. 1–9. https://doi.org/10.1016/j.envpol.2016.11.056

Glass, J.B., Orphan, V.J., 2012. Trace metal requirements for microbial enzymes involved in the production and consumption of methane and nitrous oxide. Front. Microbiol. 3, 1–20. https://doi.org/10.3389/fmicb.2012.00061

Gonzalez-Gil, G., Jansen, S., Zandvoort, M.H., van Leeuwen, H.P., 2003. Effect of yeast extract on speciation and bioavailability of nickel and cobalt in anaerobic bioreactors. Biotechnol. Bioeng. 82, 134–42. https://doi.org/10.1002/bit.10551

Guintoli, J., Agostini, A., Edwards, R., Marelli, L., 2017. Solid and gaseous bioenergy pathways : input values and GHG emissions. https://doi.org/10.2790/299090

Gustavsson, J., Shakeri Yekta, S., Sundberg, C., Karlsson, A., Ejlertsson, J., Skyllberg, U., Svensson, B.H., 2013. Bioavailability of cobalt and nickel during anaerobic digestion of sulfur-rich stillage for biogas formation. Appl. Energy 112, 473–477. https://doi.org/10.1016/j.apenergy.2013.02.009

Gustavsson, J., Svensson, B.H., Karlsson, A., 2011. The feasibility of trace element supplementation for stable operation of wheat stillage-fed biogas tank reactors. Water Sci. Technol. 64, 320–325. https://doi.org/10.2166/wst.2011.633

Harmsen, J., 2007. Measuring Bioavailability: From a Scientific Approach to Standard Methods. J. Environ. Qual. 36, 1420. https://doi.org/10.2134/jeq2006.0492

Hooda, P.S., Zhang, H., Davison, W., Edwards, A.C., 1999. Measuring bioavailable trace metals by diffusive gradients in thin films (DGT): Soil moisture effects on its performance in soils. Eur. J. Soil Sci. 50, 285–294. https://doi.org/10.1046/j.1365-2389.1999.00226.x

Houba, V.J.G., Lexmond, T.M., Novozamsky, I., Van Der Lee, J.J., 1996. State of the art and future developments in soil analysis for bioavailability assessment. Sci. Total Environ. 178, 21–28. https://doi.org/10.1016/0048-9697(95)04793-X

Jimenez, J., Aemig, Q., Doussiet, N., Steyer, J.P., Houot, S., Patureau, D., 2015. A new organic matter fractionation methodology for organic wastes: Bioaccessibility and complexity characterization for treatment optimization. Bioresour. Technol. 194, 344–353. https://doi.org/10.1016/j.biortech.2015.07.037

Jimenez, J., Gonidec, E., Cacho Rivero, J.A., Latrille, E., Vedrenne, F., Steyer, J.-P., 2014. Prediction of anaerobic biodegradability and bioaccessibility of municipal sludge by coupling sequential extractions with fluorescence spectroscopy: Towards ADM1 variables characterization. Water Res. 50, 359–372. https://doi.org/10.1016/j.watres.2013.10.048

Jimenez, J., Lei, H., Steyer, J.P., Houot, S., Patureau, D., 2017. Methane production and fertilizing value of organic waste: Organic matter characterization for a better prediction of valorization pathways. Bioresour. Technol. 241, 1012–1021. https://doi.org/10.1016/j.biortech.2017.05.176

Karlsson, A., Einarsson, P., Schnürer, A., Sundberg, C., Ejlertsson, J., Svensson, B.H., 2012. Impact of trace element addition on degradation efficiency of volatile fatty acids, oleic acid and phenyl acetate and on microbial populations in a biogas digester. J. Biosci. Bioeng. 114, 446–52. https://doi.org/10.1016/j.jbiosc.2012.05.010

Keeler, C., Kelly, E.F., Maciel, G.E., 2006. Chemical-structural information from solid-

state 13C NMR studies of a suite of humic materials from a lower montane forest soil, Colorado, USA. Geoderma 130, 124–140. https://doi.org/10.1016/j.geoderma.2005.01.015

Kögel-Knabner, I., 1997. 13C and 15N NMR spectroscopy as a tool in soil organic matter studies. Geoderma 80, 243–270. https://doi.org/10.1016/S0016-7061(97)00055-4

Kupper, T., Bürge, D., Bachmann, H.J., Güsewell, S., Mayer, J., 2014. Heavy metals in source-separated compost and digestates. Waste Manag. 34, 867–874. https://doi.org/10.1016/j.wasman.2014.02.007

Le Bars, M., Legros, S., Levard, C., Chaurand, P., Tella, M., Rovezzi, M., Browne, P., Rose, J., Doelsch, E., 2018. Drastic change in zinc speciation during anaerobic digestion and composting: instability of nano-sized zinc sulfide. Environ. Sci. Technol. acs.est.8b02697. https://doi.org/10.1021/acs.est.8b02697

Legros, S., Levard, C., Marcato-Romain, C.-E., Guiresse, M., Doelsch, E., 2017. Anaerobic Digestion Alters Copper and Zinc Speciation. Environ. Sci. Technol. 51, 10326–10334. https://doi.org/10.1021/acs.est.7b01662

Lemmon, E.W., McLinden, M.O., Friend, D.G., 2010. Thermophysical Properties of Fluid Systems, in: Linstrom, P.J., Mallard, W.G. (Eds.), National Institue of Standards and Technology. Gaithersburg MD.

Lindorfer, H., Ramhold, D., Frauz, B., 2012. Nutrient and trace element supply in anaerobic digestion plants and effect of trace element application. Water Sci. Technol. 66, 1923–1929. https://doi.org/10.2166/wst.2012.399

Maharaj, B.C., Mattei, M.R., Frunzo, L., van Hullebusch, E.D., Esposito, G., 2018. ADM1 based mathematical model of trace element precipitation/dissolution in anaerobic digestion processes. Bioresour. Technol. 267, 666–676. https://doi.org/10.1016/j.biortech.2018.06.099

Ministero delle politiche agricole alimentari e Forestali, 2015. Decreto Ministeriale del 26 Maggio 2015 - Modifiche al Decreto Legislativo n. 75/2010 e successiva modifica del 10 luglio 2013, concernente il riordino e la revisione della disciplina in materia di fertilizzanti., Gazzetta Ufficiale Serie Generale n.175 del 30-07-2015.

Ministre de l'agriculture et de l'alimentation, 2017. Arrêté du 13 juin 2017 approuvant un cahier des charges pour la mise sur le marché et l'utilisation de digestats de méthanisation agricoles en tant que matières fertilisantes, Legifrance.

Mocak, J., Bond, a. M., Mitchell, S., Scollary, G., 1997. A statistical overview of standard (IUPAC and ACS) and new procedures for determining the limits of detection and quantification: Application to voltammetric and stripping techniques (Technical Report), Pure and Applied Chemistry. https://doi.org/10.1351/pac199769020297

Moeller, K., 2015. Effects of anaerobic digestion on soil carbon and nitrogen turnover, N emissions, and soil biological activity. A review. Agron. Sustain. Dev. 35, 1021–1041. https://doi.org/10.1007/s13593-015-0284-3

Möller, K., Müller, T., 2012. Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. Eng. Life Sci. 12, 242–257. https://doi.org/10.1002/elsc.201100085

Nkoa, R., 2014. Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: A review. Agron. Sustain. Dev. 34, 473–492. https://doi.org/10.1007/s13593-013-0196-z

Odlare, M., Arthurson, V., Pell, M., Svensson, K., Nehrenheim, E., Abubaker, J., 2011. Land application of organic waste - Effects on the soil ecosystem. Appl. Energy 88, 2210–2218. https://doi.org/10.1016/j.apenergy.2010.12.043

Ortner, M., Rachbauer, L., Somitsch, W., Fuchs, W., 2014. Can bioavailability of trace nutrients be measured in anaerobic digestion? Appl. Energy 126, 190–198. https://doi.org/10.1016/j.apenergy.2014.03.070

Owamah, H.I., Dahunsi, S.O., Oranusi, U.S., Alfa, M.I., 2014. Fertilizer and sanitary quality of digestate biofertilizer from the co-digestion of food waste and human excreta. Waste Manag. 34, 747–752. https://doi.org/10.1016/j.wasman.2014.01.017

Rickard, D., Morse, J.W., 2005. Acid volatile sulfide (AVS), Marine Chemistry. https://doi.org/10.1016/j.marchem.2005.08.004

Saveyn, H., Eder, P., 2014. End-of-waste criteria for biodegradable waste subjected to biological treatment (compost & digestate): Technical proposals. https://doi.org/10.2791/6295

Scally, S., Davison, W., Zhang, H., 2006. Diffusion coefficients of metals and metal complexes in hydrogels used in diffusive gradients in thin films. Anal. Chim. Acta 558, 222–229. https://doi.org/10.1016/j.aca.2005.11.020

Scarlat, N., Dallemand, J.F., Fahl, F., 2018. Biogas: Developments and perspectives in Europe. Renew. Energy 129, 457–472. https://doi.org/10.1016/j.renene.2018.03.006

Semple, K.T., Doick, K.J., Jones, K.C., Burauel, P., Craven, A., Harms, H., 2004. Defining bioavailability and bioaccessibility of contaminated soil and sediment is complicated. Environ. Sci. Technol. 229–231.

Shakeri Yekta, S., Gustavsson, J., Svensson, B.H., Skyllberg, U., 2012. Sulfur K-edge XANES and acid volatile sulfide analyses of changes in chemical speciation of S and Fe during sequential extraction of trace metals in anoxic sludge from biogas reactors. Talanta 89, 470–477. https://doi.org/10.1016/j.talanta.2011.12.065

Shakeri Yekta, S., Hedenström, M., Stehr, J.E., Dario, M., Hertkorn, N., Björn, A., 2018. Pretreatment of anaerobic digester samples by hydrochloric acid for solution-state 1H and 13C NMR spectroscopic characterization of organic matter. Chemosphere 199, 201–209. https://doi.org/10.1016/j.chemosphere.2018.02.015

Shakeri Yekta, S., Lindmark, A., Skyllberg, U., Danielsson, Å., Svensson, B.H., 2014a.

Importance of reduced sulfur for the equilibrium chemistry and kinetics of Fe(II), Co(II) and Ni(II) supplemented to semi-continuous stirred tank biogas reactors fed with stillage. J. Hazard. Mater. 269, 83–88. https://doi.org/10.1016/j.jhazmat.2014.01.051

Shakeri Yekta, S., Svensson, B.H., Björn, A., Skyllberg, U., Yekta, S.S., Svensson, B.H., Björn, A., Skyllberg, U., 2014b. Thermodynamic modeling of iron and trace metal solubility and speciation under sulfidic and ferruginous conditions in full scale continuous stirred tank biogas reactors. Appl. Geochemistry 47, 61–73. https://doi.org/10.1016/j.apgeochem.2014.05.001

Shiva, A.H., Teasdale, P.R., Bennett, W.W., Welsh, D.T., 2015. A systematic determination of diffusion coefficients of trace elements in open and restricted diffusive layers used by the diffusive gradients in a thin film technique. Anal. Chim. Acta 888, 146–154. https://doi.org/10.1016/j.aca.2015.07.027

Simpson, A.J., McNally, D.J., Simpson, M.J., 2011. NMR spectroscopy in environmental research: From molecular interactions to global processes. Prog. Nucl. Magn. Reson. Spectrosc. 58, 97–175. https://doi.org/10.1016/j.pnmrs.2010.09.001

Soucémarianadin, L.N., Erhagen, B., Nilsson, M.B., Öquist, M.G., Immerzeel, P., Schleucher, J., 2017. Two dimensional NMR spectroscopy for molecular characterization of soil organic matter: Application to boreal soils and litter. Org. Geochem. 113, 184–195. https://doi.org/10.1016/j.orggeochem.2017.06.019

Takashima, M., Nakakihara, E., Ikemoto, R., 2018. Effects of Thermal Pretreatment and Trace Metals on High- rate Thermophilic Anaerobic Digestion of Sewage Sludge. J. Environ. Eng. 144, 3–6. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001340.

Takashima, M., Speece, R.E., 1989. Mineral nutrient requirements for high-rate methane fermentation of acetate at low SRT. Res. Journay Water Pollut. Control Fed. 61, 1645–1650. https://doi.org/10.2307/41480385

Tambone, F., Genevini, P., D'Imporzano, G., Adani, F., 2009. Assessing amendment properties of digestate by studying the organic matter composition and the degree of biological stability during the anaerobic digestion of the organic fraction of MSW. Bioresour. Technol. 100, 3140–3142. https://doi.org/10.1016/j.biortech.2009.02.012

Tambone, F., Scaglia, B., D'Imporzano, G., Schievano, A., Orzi, V., Salati, S., Adani, F., 2010. Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. Chemosphere 81, 577–583. https://doi.org/10.1016/j.chemosphere.2010.08.034

Tampio, E., Salo, T., Rintala, J., 2016. Agronomic characteristics of five different urban waste digestates. J. Environ. Manage. 169, 293–302. https://doi.org/10.1016/j.jenvman.2016.01.001

Thanh, P.M., Ketheesan, B., Yan, Z., Stuckey, D., 2017. Effect of Ethylenediamine-N,N′-disuccinic acid (EDDS) on the speciation and bioavailability of Fe2+ in the presence of sulfide in anaerobic digestion. Bioresour. Technol. 229, 169–179.

https://doi.org/https://doi.org/10.1016/j.biortech.2016.12.113

Thanh, P.M., Ketheesan, B., Yan, Z., Stuckey, D., 2016. Trace metal speciation and bioavailability in anaerobic digestion: A review. Biotechnol. Adv. 34, 122–136. https://doi.org/10.1016/j.biotechadv.2015.12.006

van Hullebusch, E.D., Guibaud, G., Simon, S., Lenz, M., Yekta, S.S., Fermoso, F.G., Jain, R., Duester, L., Roussel, J., Guillon, E., Skyllberg, U., Almeida, C.M.R., Pechaud, Y., Garuti, M., Frunzo, L., Esposito, G., Carliell-Marquet, C., Ortner, M., Collins, G., 2016. Methodological approaches for fractionation and speciation to estimate trace element bioavailability in engineered anaerobic digestion ecosystems: An overview. Crit. Rev. Environ. Sci. Technol. 46, 1324–1366. https://doi.org/10.1080/10643389.2016.1235943

Vintiloiu, A., Boxriker, M., Lemmer, A., Oechsner, H., Jungbluth, T., Mathies, E., Ramhold, D., 2013. Effect of ethylenediaminetetraacetic acid (EDTA) on the bioavailability of trace elements during anaerobic digestion. Chem. Eng. J. 223, 436–441. https://doi.org/10.1016/j.cej.2013.02.104

Vintiloiu, A., Lemmer, A., Oechsner, H., Jungbluth, T., 2012. Mineral substances and macronutrients in the anaerobic conversion of biomass: An impact evaluation. Eng. Life Sci. 12, 287–294. https://doi.org/10.1002/elsc.201100159

Wang, Y., Ding, S., Xu, D., Wang, Y., Li, Y., Gong, M., Zhang, C., 2016. Simultaneous measurements of eight oxyanions using high-capacity diffusive gradients in thin films (Zr-Oxide DGT) with a high-efficiency elution procedure. Environ. Sci. Technol. 50, 7572–7580. https://doi.org/10.1021/acs.est.6b00206

Yekta, S.S., Gonsior, M., Schmitt-Kopplin, P., Svensson, B.H., 2012. Characterization of dissolved organic matter in full scale continuous stirred tank biogas reactors using ultrahigh resolution mass spectrometry: a qualitative overview. Environ. Sci. Technol. 46, 12711–9. https://doi.org/10.1021/es3024447

Zhang, H., Davison, W., 2015. Use of diffusive gradients in thin-films for studies of chemical speciation and bioavailability. Environ. Chem. 12, 85–101. https://doi.org/10.1071/EN14105

Zhang, H., Davison, W., 1999. Diffusional characteristics of hydrogels used in DGT and DET techniques. Anal. Chim. Acta 398, 329–340. https://doi.org/10.1016/S0003-2670(99)00458-4

Zhang, H., Davison, W., 1995. Performance Characteristics of Diffusion Gradients in Thin Films for the in Situ Measurement of Trace Metals in Aqueous Solution. Anal. Chem. 67, 3391–3400. https://doi.org/10.1021/ac00115a005

Zhang, H., Davison, W., Knight, B., Mcgrath, S., 1998. In situ measurements of solution concentrations and fluxes of trace metals in sells using DGT. Environ. Sci. Technol. 32, 704–710. https://doi.org/10.1021/es9704388

Zhang, W., Zhang, L., Li, A., 2015. Enhanced anaerobic digestion of food waste by trace metal elements supplementation and reduced metals dosage by green chelating

agent [S, S]-EDDS via improving metals bioavailability. Water Res. 84, 266–277. https://doi.org/10.1016/j.watres.2015.07.010

Zhu, N., Li, Q., Guo, X., Zhang, H., Deng, Y., 2014. Sequential extraction of anaerobic digestate sludge for the determination of partitioning of heavy metals. Ecotoxicol. Environ. Saf. 102, 18–24. https://doi.org/10.1016/j.ecoenv.2013.12.033

Zufiaurre, R., Olivar, A., Chamorro, P., Nerin, C., Callizo, A., 1998. Speciation of metals in sewage sludge for agricultural uses. Analyst 123, 255–259. https://doi.org/10.1039/a705168i

ORIGINAL PAPERS

I

A SIMULTANEOUS ASSESSMENT OF ORGANIC MATTER AND TRACE ELEMENTS BIO-ACCESSIBILITY IN SUBSTRATE AND DIGESTATE FROM AN ANAEROBIC DIGESTION PLANT

by

Andreina Laera, Sepehr Shakeri Yekta, Mattias Hedenström, Rémy Buzier, Gilles Guibaud, Mårten Dario, Giovanni Esposito & Eric D. van Hullebusch,
2019

Bioresource Technology, Submitted for publication

A simultaneous assessment of organic matter and trace elements bio-accessibility in substrate and digestate from an anaerobic digestion plant

Authors and affiliation

Andreina Laera[a,d,*], Sepehr Shakeri Yekta[b], Mattias Hedenström[c], Rémy Buzier[d], Gilles Guibaud[d], Mårten Dario[b], Giovanni Esposito[e], Eric D. van Hullebusch[f]

[a] University of Paris-Est, Laboratoire Géomatériaux et Environnement (EA 4508), UPEM, 77454 Marne-la-Vallée, France, andreina.laera@u-pem.fr or piedelibero89@gmail.com

[b] Department of Thematic Studies-Environmental Change and Biogas Research Center, Linköping University, 581 83 Linköping, Sweden

[c] Department of Chemistry, Umeå University, 901 87 Umeå, Sweden

[d] University of Limoges, PEIRENE, Equipe Développement d'indicateurs ou prévision de la qualité des eaux, URA IRSTEA, 123 Avenue Albert Thomas, 87060 Limoges Cedex, France

[e] University of Napoli "Federico II", Department of Civil, Architectural and Environmental Engineering, via Claudio 21, 80125 Napoli, Italy

[f] Université de Paris, Institut de Physique du Globe de Paris, CNRS, UMR 7154, F-75238 Paris, France

*Corresponding author: andreina.laera@u-pem.fr

Abstract

This study evaluates a simultaneous assessment of organic matter (OM) and trace elements (TE) bio-accessibility in substrate and digestate from a full-scale anaerobic digester by a sequential OM extraction method. Simultaneous release of TE was determined along with the extraction of different OM fractions and the effects of extracting reagents on characteristics of OM were evaluated by nuclear magnetic resonance (NMR) spectroscopy. The reagents used for sequential extraction of OM were not enough selective. However, proteins were particularly removed by 0.1 M NaOH, while 72% H_2SO_4 mainly extracted hemicellulose and cellulose. The OM fractionation allowed for simultaneous extraction of >60% of total As, Cd, Co, Fe, Mn, Ni and Zn, while the extraction efficiency was limited for Al, Cr, Cu, Mo, and Pb. In substrate, >50% of total As, Co, Mn and Ni and <40% of total Fe, Zn and Mo were identified in bio-accessible fractions. In digestate, all elements demonstrated poor bio-accessibility except for As.

Keywords

Sequential chemical extraction

Organic matter fractionation

Trace elements fractionation

NMR spectroscopy

Anaerobic co-digestion

1. Introduction

Availability of trace elements (TE) as micronutrients is essential for stable and efficient performance of anaerobic digestion processes (Feng et al., 2010; Karlsson et al., 2012; Lindorfer et al., 2012). Due to a complex network of reactions, controlling the chemical speciation of TE, a small fraction of TE in the digesters environment is often available for microbial activities, while the majority of TE occur as precipitates, adsorbed or complexed species (Aquino and Stuckey, 2007; Maharaj et al., 2019, 2018). Several studies attempted to determine the fraction of TE available for uptake by microorganisms with sequential extraction procedures, such as the modified Tessier method (van Hullebusch et al., 2005) and the Community Bureau of Reference (BCR) method (Rauret et al., 1991) to guarantee an adequate supply of TE in digesters (Braga et al., 2017; Cao et al., 2018; Ortner et al., 2014). The procedure involves sequential treatment of the samples by chemical reagents such as neutral salts, weak and strong acids or reducing and oxidizing agents to selectively extract TE. Despite the limitations associated to the sequential extraction procedures including the poor selectivity of chemical reagents (Bacon and Davidson, 2008; Filgueiras et al., 2002), the lack of uniformity in the procedure and the complexity of chemical composition of anaerobic samples (Thanh et al., 2016), there is a growing interest in using sequential extraction procedures to fractionate TE (Ortner et al., 2014; Zhu et al., 2014). Indeed, results obtained from sequential extraction methods are valuable for anaerobic digestion processes and for environmental risk assessments of digestate utilization as a soil fertilizer (Ortner et al., 2014; Zhu et al., 2014), as it allows to quantitatively determine the TE fractions with different degree of solubility and reactivity. Moreover, sequential extraction methods are useful tools to assess mobility, accessibility, and potential bio-availability of TE (Harmsen, 2007). Braga et al. (2017) observed that micronutrient TE, including Se, Zn, Ni and Fe were

mainly found in the organic matter (OM)/sulfide fraction of the modified Tessier method applied on sewage sludge samples. Similar results were also found by Zufiaurre et al. (1998) in sewage sludge samples. Such fraction is considered less mobile than other TE fractions extracted by the sequential extraction procedure and less bio-accessible for microbial uptake (Filgueiras et al., 2002).

Different chemical interactions between TE and organic/inorganic compounds originating from the substrate and generated during the anaerobic digestion process will determine the chemical speciation of TE and consequently their bio-accessibility and bioavailability for anaerobic microorganisms in digesters and for plants and soil microorganisms when digestate is spread on lands (Fermoso et al., 2015; Thanh et al., 2016). Inorganic compounds such as sulfide (S^{2-}), phosphate (PO_4^{3-}) and carbonate (CO_3^{2-}) may precipitate TE in anaerobic digestion systems as simulated by Maharaj et al. (2018) with a dynamic mathematical model based on anaerobic digestion model no.1 (ADM1). Such inorganic compounds would compromise the availability of TE for microbial uptake in anaerobic digesters (Callander and Barford, 1983). In addition, TE could be complexed with organic chelators becoming either more or less available for microbial uptake depending on the binding strength of metal-organic complexes. Organic compounds contain functional groups such as carboxyl, hydroxyl or amino groups with high affinity to complex with TE (Callander and Barford, 1983). Gonzalez-Gil et al. (2003) observed that amino acids in yeast extracts form soluble complexes with Ni and Co which prevent their precipitation with sulfide and consequently increased their availability for microorganisms. Moreover, the degradation process of bio-accessible OM deriving from substrate or digestate, can release TE in solution that becomes potential bio-accessible for up-take by anaerobic digester microorganisms or soil-dwelling organisms (Knoop et al., 2018). Accordingly, association of TE with OM play an important role in bioavailability of TE.

Jimenez et al. (2017, 2014) assessed the bio-degradability and the bio-accessibility of OM in organic wastes using a physical-chemical sequential extraction procedure for a large number of samples, including municipal sludge samples, municipal solid wastes, digestate and compost. The sequential extraction procedure includes the following fractions: i) Extractable Soluble from Particulate Organic Matter (SPOM), which contains water-soluble proteins and sugars; ii) Readily Extractable Organic Matter (REOM), representing easily accessible proteins and lipids; iii) Slowly Extractable Organic Matter (SEOM), containing humic-like and fulvic acid-like structures as well as complex proteins and certain lignocellulosic compounds and iv) Poorly Extractable Organic Matter (PEOM), targeting hemicellulose and cellulose (Jimenez et al., 2017). The development of OM fractionation methods allows assessing the accessibility of OM as carbon and energy sources for microorganisms in anaerobic digesters. However, to the best of our knowledge, no research work assessed simultaneous OM and TE extraction for assessment of the bio-accessibility of a combined source of carbon, energy and micronutrient TE.

Thus, we aim to evaluate the application of a sequential extraction method adapted for OM fractionation by Jimenez et al. (2017, 2014) to determine simultaneously the accessibility of TE in substrate and digestate samples. For this purpose, substrate and digestate samples from a full-scale anaerobic digester, regularly supplied with TE supplements, were used to determine the TE concentrations in different OM fractions. The TE investigated in this study are Co, Fe, Ni, Mn, Mo, and Zn, which are important micronutrients for microbial activities (Gustavsson et al., 2013, 2011) and Al, As, Cd, Cr, Cu and Pb, which could be harmful to soil microorganisms and plant growth once digestate is applied as soil amendment (Bajgiran, 2013; Kupper et al., 2014; Nkoa, 2014). Moreover, changes in structural characteristics of OM after each step of the extraction procedure were studied by nuclear magnetic resonance (NMR) spectroscopy in order to assess

different organic groups in the samples, which were removed by extracting reagents during the sequential extraction procedure.

2. Material and methods

2.1. Samples

Substrate and digestate were collected from a full-scale anaerobic co-digestion plant located in Linköping, Sweden. The anaerobic digestion plant has a capacity of 125 000 tons of waste per year and treats the organic fraction in household waste (50%), slaughterhouse waste (25%) and industrial waste (25%) at 42°C with an organic loading rate of 4,5 kgVS/L·d and a hydraulic retention time of 37 days. The substrate was collected from a tank after 1-hour pasteurization at 70°C and TE addition, whereas the digestate was collected from the main anaerobic digester sampling port. About 1 liter of each sample was collected in acid washed polypropylene (PP) bottles. The bottles were flushed with nitrogen (N_2) prior to sampling and closed with a lid after collection to reduce sample exposure to air during sampling and transportation from the plant to the laboratory. Once in the laboratory, the samples were immediately treated in accordance to the sequential extraction procedure.

2.2. Sequential extractions procedure

The sequential extractions of dissolved organic matter (DOM), REOM and SEOM were carried out as described by Jimenez et al. (2014), while SPOM and PEOM fractions were extracted according to Jimenez et al. (2017). The latter modified protocol includes calcium chloride ($CaCl_2$) reagent for SPOM extraction and sulfuric acid (H_2SO_4) for PEOM extraction compared to the procedure proposed by Jimenez et al. (2014). Moreover, we slightly modified the protocols to adapt the method for simultaneous extraction of OM and TE (Table 1). The main modifications involve the use of raw sample, rather than freeze dried sample, N_2 flushing during operations to reduce sample oxidation and changes in TE speciation (*e.g.* formation of metal

95 oxides (Ortner et al., 2014)) which would determine a change in the bio-accessibility pattern of
96 trace elements. Moreover, the mass of the sample and the volume of reagents were decreased
97 compared to the original procedures to adapt the method to facilities available in the laboratory
98 such as the high-speed centrifuge (Beckman J2-21M, USA), which was used to separate the
99 supernatants from the solids. In short, the first step of the procedure separates DOM from the
100 solid residue. Approximately 300-600 ml of sample, with a total solids content of 4.8±0.2 wt%,
101 and 14.6±0.1 wt% for digestate and substrate, respectively, was centrifuged at 18600×g for 30
102 min at 10°C. Then, the supernatant, containing DOM, was filtered through 0.45 µm
103 polyethersulfone (PES) syringe filters (Pall Laboratory). The solid residue was flushed with N_2,
104 sealed and stored at 4°C in PP centrifuge tubes (Sarstedt) before performing the next extraction
105 step. In the second step, SPOM was extracted according to the procedure by Jimenez et al.
106 (2017). Approximately 3 g of pellet were shaken in polypropylene copolymer tubes (Termo
107 Scientific Nalgene) with 24 ml (mass ratio 1:8) of 10 mM $CaCl_2$ (pH 8) at 200 rpm and at 30°C
108 for 15 min. The suspension was then centrifuged at 18600×g for 30 min at 4°C and the
109 supernatant containing SPOM was recovered and filtered through 0.45 µm PES syringe filters.
110 The residual solid was treated with the same reagent three more times. During extraction of
111 SPOM, N_2 was flushed in the tubes. Subsequently, the solid residue was rinsed four times with 24
112 ml of 10 mM NaCl and 10 mM NaOH (pH 11) (Jimenez et al., 2014). The suspension was
113 shaken, centrifuged and filtered to recover REOM fraction. Thereafter, the residual pellet was
114 used to extract carbonate, sulfides and hydroxides (CSH) fraction by adding 24 ml of 0.1 M HCl
115 for 1 h at 30°C and 200 rpm (Jimenez et al., 2014). Unlike the original procedure (Jimenez et al.,
116 2014), this fraction was recovered for further analyses. The resulting solid residue was washed
117 with ultrapure water and neutralized to pH 7 with 0.1 M NaOH. Subsequently, the solid residue
118 was suspended in 24 ml of 0.1 M NaOH (pH 12) and shaken at 200 rpm and at 30°C for 1 h to

recover the SEOM fraction (Jimenez et al., 2014). This step was repeated three more times. Finally, the residual pellet was shaken two times with 24 ml of 72% (w:w) H_2SO_4 for 3 h at 30°C and 200 rpm for extraction of PEOM (Jimenez et al., 2017). The residual solid, which is the Non-Extractable Organic Matter (NEOM), was recovered and freeze-dried for further analyses. The sequential extraction was performed on triplicate samples. All reagents were prepared in acid washed glassware and with ultrapure deaerated water.

Table 1 shows the sequential extraction steps of the procedure.

(Table 1 here)

2.3. Analytical procedures

2.3.1. Chemical analysis

The pH of the samples was measured with a pH meter (InoLab 7310, WTW, Weilheim, Germany). Total solids (TS) and volatile solids (VS) content in the raw samples and pellet collected after DOM extraction were quantified in triplicates according to the Swedish Standard method (SS-028113; 25). Thus, an aliquot of sample was dried in porcelain crucibles at 105 °C for 20 h to measure TS content. Then, the dried samples were heated up to 550°C for 2 h in a muffle furnace to determine VS content. Dissolved organic carbon (C) was measured in the filtered supernatants by total organic carbon analyzer (TOC-VCHS, Shimadzu, Japan). During analysis, ultrapure water was analyzed after each set of triplicates to avoid cross contamination.

Total C and N content in the solid residue collected at each extraction step was determined by CHNS/O elemental analyzer (EA2400, Perkin Elmer, USA). Prior to analysis, the samples were freeze-dried and finely ground with a mortar and pestle.

140 The concentration of TE for each extracted faction was quantified in the filtered supernatants,
141 whereas the total TE' content (*i.e.* Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb and Zn) was
142 extracted after digestion of substrate and digestate samples according to the Swedish standard
143 method (SS028311) using 7 M HNO_3 in an autoclave at 120 °C for 30 min as described by
144 Shakeri Yekta et al. (2014a). Trace element concentrations in the samples collected both after the
145 acid digestion method and the sequential extraction procedure were quantified by inductively
146 coupled plasma mass spectrometry (ICP-MS, Nexion 300D, Perkin Elmer, USA). Before ICP-
147 MS analysis, if required, samples were acidified with concentrated HNO_3 (1% of the sample
148 volume) and stored at 4°C until analysis. To reduce possible chemical interference, some of the
149 TE were analyzed by using kinetic energy discrimination (KED) or dynamic reaction cell (DRC)
150 mode during ICP-MS analysis. A graphical scheme of the analyses performed on the collected
151 samples is showed in Figure 1.

152 (Figure 1 here)

153 2.3.2. Nuclear magnetic resonance spectroscopy

154 NMR analysis was performed on the solid residues recovered at each step of the extraction
155 procedure. Solid residues were preferred to the liquid fractions to reduce possible interferences,
156 generated by the chemical reagents, with the sample NMR signals.

157 About 0.4 g dry mass of sample was pre-treated with 2 M HCl for 1 h to remove the
158 paramagnetic TE that would be detrimental to the quality of the NMR spectra according to
159 Shakeri Yekta et al. (2018). The suspension was centrifuged and the supernatant was discarded,
160 while the solid residue was recovered and freeze-dried. Approximately 80 mg of each sample was
161 transferred to 4 mm ZrO_2 rotors for solid state cross polarization magic angle spinning (CPMAS)
162 ^{13}C NMR analysis and approximately 100 mg of sample was milled using a Fritsch Pulverisette 7

planetary ball-mill to prepare it for solution-state 1D ^{1}H and 2D ^{1}H-^{13}C heteronuclear single quantum coherence (HSQC) NMR analysis. The protocol used for grinding consisted of 5 × 10 min milling with 5 min pauses in between to prevent overheating of the samples. 20 mg of milled sample were transferred to 5 mm NMR tubes and 600 µl of deuterated dimethyl sulfoxide (DMSO-d_6) was added. The CPMAS ^{13}C analysis was performed in triplicate and these were later pooled to get sufficient material for liquid state NMR analysis.

Solid state CPMAS ^{13}C NMR spectra and liquid state 1D ^{1}H and 2D HSQC ^{1}H-^{13}C NMR spectra were acquired using a Bruker 500 MHz AVANCE III spectrometer equipped with a 4 mm MAS probe and a Bruker 600 MHz AVANCE III HD spectrometer equipped with a 5 mm cryoprobe, respectively. CPMAS ^{13}C NMR spectra were recorded using cp pulse sequence and 3500 scans. The relaxation delay was 1 s and spin-rate was 10 kHz. 1D ^{1}H and 2D HSQC ^{1}H-^{13}C NMR spectra were recorded using zg30 and hsqcetgpsisp2.2 pulse sequences and 8 and 16 scans, respectively. The relaxation delay was 1.5 s and 2 s for 1D ^{1}H and 2D HSQC ^{1}H-^{13}C NMR, respectively. The spectra processing was performed in Topspin 3.5 (Bruker Biospin, Germany) and spectra were calibrated using adamantane as an external reference for CPMAS spectra or the residual DMSO peak ($\delta_{H/C}$: 2.49/39.5 ppm) in the case of 2D ^{1}H-^{13}C HSQC spectra.

2.4. Analytical quality control

The methodological limit of detection (MLD) and quantification (MLQ) were calculated using the conservative formula as the average plus three or ten times the standard deviation of the blanks, respectively, to consider contamination from elements in the reagents. During the sequential extraction procedure, we used 36 and 96 procedural blanks for TE and dissolved organic C analysis, respectively. Quality controls at 20 and 50 mg/L dissolved organic C were analyzed for every 10 samples during dissolved organic C analysis. The recovery was equal or

above 94% among all analyses. A reference material Sewage sludge CRM029-050 was acid digested in triplicate to evaluate the performance of the adopted total acid digestion method for TE' analysis. The accuracy of the method, calculated by dividing the mean observed concentration to the certified value (van Reeuwijk and Houba, 1998), was equal or higher than 90% for all analyzed elements.

In addition, spiked samples were analyzed to check possible matrix interferences on the measured concentrations of analytes due to the diversity of reagents used to perform the sequential extraction procedure. The method adopted to evaluate the matrix effect is standard addition method. We observed no significant matrix effect for dissolved organic C analysis ($p \geq 0.04$) in all OM fractions, whereas we observed matrix effect for TE analysis in PEOM fraction extraction and therefore results for this fraction are not presented.

3. Results and discussion

3.1. Organic matter composition of substrate and digestate

The TS content of substrate and digestate samples were 14.6±0.1 and 4.8±0.2 wt%, respectively, with the VS contents of 91.4±0.4 and 76.2±0.6 % of TS. The TS content after centrifugation and separation of the liquid fraction (*i.e.* solid pellets used for the sequential extraction procedure) were 33.1±0.4 wt% for substrate and 19.6±0.5 wt% for digestate samples. The pH of substrate and digestate was 4.9 and 8.1, respectively. We observed different distribution of C among the operationally defined organic fractions (*i.e.* DOM, SPOM, REOM, SEOM and PEOM) for substrate and digestate samples (Figures 2a and 2b). A large proportion of organic C in the substrate was present as DOM (76% of extracted organic C), whereas PEOM had the highest proportion among the OM fractions of the digestate (47% of extracted organic C). The DOM fraction mainly contains water-soluble organic substances, whereas PEOM contains recalcitrant and insoluble organic compounds according to Jimenez at al. (2017, 2014). In substrate, only 9% of extracted organic C was contained in PEOM and 15% of extracted organic C was present in SPOM, REOM and SEOM, which mainly contain proteins and sugars, lipids, humic-like and fulvic acid-like structures based on fluorescence spectroscopic characterization of the OM extracted in the supernatant after each step by Jimenez et al. (2017). In digestate, 28% of extracted organic C was as DOM, while 25% of extracted organic C was present in SPOM, REOM and SEOM.

To support the OM characterization of the extracted fractions provided by Jimenez et al. (2017, 2014), NMR spectroscopy was applied to look into the structural compositions of the organic molecules in the solid residues recovered after each extraction step of the sequential extraction

219 procedure. Moreover, NMR spectroscopy has shown to yield good resolutions of spectra in
220 complex organic matrices such as substrate and digestate (Shakeri Yekta et al., 2018).

221 Based on Kögel-Knabner (1997) and on Tambone et al. (2009), ^{13}C CPMAS NMR spectra,
222 presented in Figures 2c and 2d , were divided in five regions corresponding to different organic
223 structures: aliphatic chain C (δ_C 0-47 ppm), carbohydrates (δ_C 47-90 ppm), anomeric C (δ_C 90-
224 110 ppm), aromatic C (δ_C 110-160 ppm) and carbonyl C (δ_C 160-187 ppm). Figures 2c and 2d
225 show a higher contribution of aliphatic, aromatic and carbonyl C resonances in spectra of the
226 digestate compared to the substrate, while carbohydrates signals had a higher contribution in the
227 substrate spectra. Accordingly, aliphatic, aromatic and carbonyl C, mainly attributed to lipid-
228 and/or protein-like structures (Keeler et al., 2006; Kögel-Knabner, 1997; Simpson et al., 2011),
229 were enriched in the solid phase of the digestate upon anaerobic digestion. These observations are
230 in agreement with previously reported ^{13}C CPMAS NMR results by Tambone et al. (2013), who
231 showed a decrease of O–alkyl carbon signals (δ_C 47-113 ppm) attributed to carbohydrates in
232 substrates, whereas aromatic-C (δ_C 113–160 ppm) and aliphatic chain C (δ_C 0– 47 ppm)
233 accumulated in digestate samples.

234 Additionally, 2D ^{1}H-^{13}C HSQC NMR spectra of solid residue from substrate and digestate show
235 that anomeric signals at δ_H 4.45-5.2 ppm and δ_C 99-102 ppm (Simpson et al., 2011) as well as O-
236 alkyl signals at δ_H 3.4-3.8 ppm and δ_C 68-79 ppm (Soucémarianadin et al., 2017) from
237 hemicellulose and starch were more readily degraded during the anaerobic digestion process
238 compared to non-anomeric (δ_H 3.24-3.63 ppm and δ_C 60-72 ppm) (Soucémarianadin et al., 2017)
239 and anomeric C signals ($\delta_{H/C}$: 4.32/102.4 ppm) (Soucémarianadin et al., 2017) from cellulose,
240 which was left in the digestate. Interestingly, the peak from unsaturated double bonds of aliphatic
241 structures such as fatty acids ($\delta_{H/C}$: 5.3/130.1 ppm) is not visible in the digestate sample,

242 indicating that aliphatic double bonds in substrate OM were susceptible to degradation during the
243 anaerobic digestion.

244 (Figure 2 here)

245 3.2. Structural characteristics of sequentially extracted organic matter fractions

246 NMR spectroscopy analyses of the solid pellets after each step of the sequential extraction
247 allowed to differentiate the major structural groups, which remain after application of chemical
248 reagents used for fractionation of particulate OM (Table 1).

249 Additional results display that the solid residues recovered after DOM, SPOM, REOM and
250 SEOM extractions have a comparable distribution of C among different organic groups in both
251 substrate and digestate samples. Only a slight reduction of carbohydrates was observed in the
252 SEOM solid residue in substrate. On the other hand, we observed a reduction of C in anomeric
253 and carbohydrate organic groups in solid residues of substrate and digestate after the PEOM
254 extraction, implying that application of 72% H_2SO_4 resulted in partial dissolution of cellulosic
255 structures.

256 The ^{1}H-^{13}C HSQC NMR spectra of digestate solid residues collected after extraction of DOM,
257 SPOM, and REOM fractions were qualitatively similar. Thus, OM extraction by 10 mM $CaCl_2$
258 and a mixture of 10 mM NaCl and 10 mM NaOH, used for extraction of SPOM and REOM, did
259 not selectively remove OM groups from the particulate OM. However, the ^{1}H-^{13}C HSQC NMR
260 spectrum of pellet collected after extraction of SEOM fraction contained fewer cross peaks within
261 the chemical shift regions assigned to CH(α) groups of amino acids in peptide chains and proteins
262 ($\delta_{H/C}$: 4.0-4.7/45-62 ppm) (Simpson et al., 2011). Furthermore, several peaks in the aliphatic
263 region where signals from amino acid side-chains appear, e.g. the peak at $\delta_{H/C}$: 2.0/15.1 ppm

assigned to methionine CH_3-groups (Shakeri Yekta et al., 2018), were absent or had reduced intensities in the spectra of pellets after SEOM extraction. The major peaks in the aromatic region, $\delta_{H/C}$: 6.5-7.4/113-134 ppm, assigned to aromatic side-chains of amino acids (based on comparisons with reference spectra) also experienced a reduction in signal intensities after SEOM extraction. Accordingly, 0.1M NaOH reagent mainly extracted proteins in SEOM fraction. It is notable that the presence of protein-derived resonances in the spectra even after SEOM extraction indicates that the proteins were only partially extracted during this step.

The ^{1}H-^{13}C HSQC NMR spectra of digestate proved that carbohydrate signals mainly from cellulose, $\delta_{H/C}$: 4.1-5.2/94-106 ppm (anomeric) and $\delta_{H/C}$: 2.9-4.11/59-84 ppm (O-alkyl), and signals related to amino acids were removed from the solid residue after the PEOM extraction. Thus, the OM extracted by 72% H_2SO_4 mainly originate from carbohydrate and partially from protein contents of the samples. These results support the findings in Jimenez et al. (2015), where the authors identified the biochemical nature of each extracted fractions by testing the sequential extraction protocol on several representative samples (e.g. lipid-rich agri-food waste, cardboard and crispbread). Based on percentage of chemical oxygen demand (COD) extracted in each fraction from the representative samples, the authors found that protein-like and lipid-like compounds were mainly extracted in SEOM fraction, whereas carbohydrates and holocelluloses in the PEOM fraction.

Similarly, ^{1}H-^{13}C HSQC NMR spectra of substrate solid residues, collected after extraction of DOM, SPOM, and REOM were qualitatively similar, whereas peaks from CH(α) groups of the amino acids and peaks from aromatic amino acid side-chains were significantly reduced in solid residue collected after SEOM fraction. Moreover, signals related to cellulose and amino-acids were removed from the solid residue of substrate after the PEOM extraction.

287 Furthermore, comparison of the amount of C extracted during the sequential extraction procedure
288 and total C content of the samples demonstrated that more than 60% of the total initial C was still
289 retained in both the substrate and digestate samples after sequential extraction. Nevertheless, the
290 structural characterization of OM in our study revealed that NEOM, representing the fraction
291 with low degree of bio-accessibility (Jimenez et al., 2015), comprised mainly of aliphatic and
292 aromatic CH groups of the protein/biomass fraction of the OM.

293 The major organic molecules targeted by the chemical reagents used for fractionation of
294 particulate OM according to Jimenez et al. (2017, 2015, 2014) and the findings of this study are
295 summarized in Table 2. It should be highlighted that in this study, NMR spectroscopy was
296 performed on the solid residues recovered at each step of the extraction procedure, whereas
297 Jimenez et al. (2017, 2015, 2014) identified the nature of the organic molecules in the liquid
298 fractions extracted by the fractionation procedure. Similarities are only found in SEOM and
299 PEOM fractions, whereas the organic molecules found in NEOM fraction reflects the nature of
300 substrate analyzed.

301 (Table 2 here)

302 ## 3.3. Simultaneous extraction of trace elements with organic matter fractions

303 Concentrations of TE extracted at each step during sequential extraction of OM are reported in
304 Table 3. Element contents in CSH fraction are also reported as this fraction may include TE
305 likely bound to inorganic ligands. Quantification of TE concentrations in PEOM fraction had a
306 high degree of uncertainty due to analytical interferences, caused by reagent matrix and are
307 omitted from Table 3.

308 Overall, we observed a higher concentration of total TE in digestate than substrate (on TS basis),
309 which is also a result of OM content reduction in digestate compared to substrate. Among

310 elements, total concentrations of Fe, Al, Mn and Zn prevailed in digestate and substrate, whereas
311 total concentration of As, Cd, Co, Cr, Mo, and Pb were lower than 11 µg/gTS_{in} in both samples.

312 (Table 3 here)

313 More than 60% of total Cd, Co, Fe, Mn, Ni and Zn were extracted together with DOM, SPOM,
314 REOM, CSH and SEOM organic fractions of the digestate and substrate samples, whereas it is
315 assumed that the remaining concentrations were in the residual pellet after SEOM fraction
316 extraction. Additionally, sum of As concentrations in the fractions extracted along with
317 sequential extraction of OM from substrate was higher than the As concentrations measured after
318 total digestion of the samples by 7M HNO_3 (Table 3). Molybdenum concentration in the samples
319 was relatively low (2.3±0.1 µg/gTSin in digestate, 0.68±0.02 µg/gTSin in substrate) and only
320 13% of total Mo was recovered during the sequential extraction procedure, primarily in DOM
321 and SPOM fraction. The recovery of Cr was also low, *i.e.* 18% and 29% for digestate and
322 substrate, respectively, mainly found in DOM and CSH fractions, whereas only 4% of Cu was
323 recovered in DOM fraction in digestate. In general, the highest concentration of all TE was found
324 in DOM and CSH fractions of digestate and substrate. Low concentrations of Al, Fe, Mn, Mo and
325 Ni were found in SPOM fraction of substrate and digestate, additionally Co and Zn were
326 extracted from substrate in SPOM fraction. Notably, the concentration of five elements was
327 below MLD and MLQ in digestate and substrate. Among quantified elements, Co, Fe, Mn and
328 Mo were found in REOM fraction of both samples, additionally Al and Ni were extracted from
329 digestate in this fraction. Finally, SEOM contained Al, Cr, Fe, Mn, Ni and Zn with relatively low
330 concentrations extracted from both samples.

331 It should be emphasized that, except for the DOM fraction obtained by centrifugation, the other
332 extraction steps involve reagents that may interact with TE species in the sample and promote the

dissolution/precipitation of elements together with OM extraction. However, we do not exclude that TE, which were extracted together with the operationally defined fractions of OM, may originate from organically-bound and/or inorganic TE compounds (*e.g.* CSH fraction) in the samples. To further assess the origin of TE in OM fractions and assess the contribution of TE containing minerals compounds during the sequential extraction, we performed the CSH fraction extraction step between DOM and SPOM extraction steps. Addition of 0.1M HCl during CSH extraction results in dissolution of metals bound to minerals under acidic conditions *e.g.* metals bound to carbonate, phosphate and amorphous metal sulfide (Albacete et al., 2015; Filgueiras et al., 2002; Rickard and Morse, 2005). Thus, shifting the extraction of CSH fraction prior to sequential extraction of SPOM, REOM, and SEOM allows the removal of metals bound to minerals, whereas TE simultaneously extracted during the subsequent extraction steps represent the fractions most likely bound to OM. Thus, concentrations of TE in each fraction provide information on potential association of elements with operationally defined OM fractions. Indeed, we noticed that the concentration of elements found in the "shifted" CSH fraction is similar to the concentration of elements found in CSH fraction of the original fractionation procedure. Accordingly, the assessment of simultaneous extraction of OM and TE suggested that 31% to 98% and from 61% to 94% of total elements' content, depending on the specific element, are associated with the mineral fraction (*i.e.* CSH fraction) (or strongly bound to organic compounds) in substrate and digestate, respectively, whereas the remaining portion is likely associated with the extracted OM fractions.

3.4. Implications for simultaneous assessment of trace elements and organic matter bio-accessibility

Comparison of the OM fractionation of digestate and substrate demonstrated that the anaerobic digestion process resulted in a decrease of dissolved organic C in DOM fraction of the substrate, while the PEOM fraction was enriched in the digestate (Figure 2Figure a, b). The DOM fraction contains more bio-accessible organic substances compared to the other fractions, whereas PEOM represents the least bio-accessible fraction of the OM, which is mainly composed of hemicellulose, cellulose and starch based on ^{13}C CPMAS and ^{1}H-^{13}C HSQC NMR spectroscopy in this study (Table 2).

A distribution of TE based on different degree of bio-accessibility is proposed in Figure 3Figure. The proposed distribution is based on the knowledge of the leaching strength of the reagents used during the extraction procedure and the results obtained from the modified sequential extraction procedure (*i.e*. mineral fraction extracted at the beginning of the extraction procedure). Therefore, we suggest that TE found in DOM fraction are mobile in the digester environment and thus, more bio-accessible. The TE found in SPOM fraction are potentially bio-accessible, whereas TE found in REOM, CSH and SEOM fractions are considered poorly-bio-accessible. It is noteworthy that the CSH fraction contains metals bound to minerals with different solubility (e.g. metals bound to phosphate and carbonate minerals encompass a higher solubility and potential bioavailability in the digester environment than amorphous metal-sulfides). Moreover, part of TE extracted in CSH fraction could be associated to mineral particles present in extracellular polymeric substances (EPS) as observed by D'Abzac et al. (2010) using scanning electron microscopic analysis in anaerobic granular sludge. Therefore, association of metals in CSH to poorly-accessible fraction should be considered relative to the operationally-defined fractions of metals in this study, which

376 is based on leaching strength of the reagent used. Finally, the elements not extracted by the
377 sequential extraction procedure are likely not immediately bio-accessible, but they may be
378 mobilized on the long term after degradation of the OM present in PEOM and NEOM fractions.

379 The high concentration of TE found in DOM fraction is related to the presence of dissolved metal
380 species (free ions and complexes with inorganic and organic metal-binding ligands) as well as
381 metal-containing colloids and particles (<0.45 μm). Organic macromolecules such as proteins
382 may as well contain metals (e.g. Co-containing vitamin B12), which contribute to the pool of
383 metals associated with DOM (Shakeri Yekta et al., 2014a; Zhu et al., 2014). Therefore, we
384 hypothesize that TE in DOM fraction are accessible for interaction with the biological interface.
385 Regarding TE found in SPOM fraction, obtained by washing the sample pellets with $CaCl_2$
386 reagent, we assume that elements are potentially mobile and bio-accessible since they were likely
387 released in solution by ion exchange mechanisms with Ca^{2+} or Cl^-, therefore TE bio-accessibility
388 is related to availability of this fraction for taking part in ion-exchange reactions. Indeed, $CaCl_2$
389 reagent is commonly used in soil analysis to extract the exchangeable fraction of TE which is
390 also the most available fraction for plant uptake (Filgueiras et al., 2002; Houba et al., 1996). The
391 TE associated with CSH fraction is likely related to the TE as minerals, such as amorphous metal
392 sulfide, metal carbonate and metal phosphate precipitates, which are dissolved under acidic
393 conditions upon addition of HCl. Accordingly, the accessibility of TE in the form of inorganic
394 precipitates in solid phase is probably limited, and the availability of TE bound to this fraction is
395 largely dependent on the solubility of the metal-containing minerals. Chemical speciation
396 analysis of TE in different anaerobic digesters suggested that sulfide is likely the major inorganic
397 ligand, scavenging TE from aqueous phase in co-digesters and the TE in solid phase is dominated
398 as TE-sulfide (Shakeri Yekta et al., 2014b). Due to poor solubility of TE-sulfide minerals as
399 potential dominant species in the CSH fraction, the accessibility of metals in this fraction for

400 microorganisms is likely constrained. REOM and SEOM fractions include elements extracted
401 from the samples under alkaline condition (pH 11-12). Prevalence of such high pH is uncommon
402 in anaerobic digesters and in environment. Furthermore, dissolution of metal species, which
403 commonly occur at low pH, is unlikely to occur during extraction of REOM and SEOM
404 fractions, implying that TE extracted might potentially originate from the simultaneously
405 extracted OM. As this fraction of OM is considered less accessible and could be solubilized only
406 after a pH increase, the bio-accessibility of TE associated with REOM and SEOM fractions may
407 be limited.

408 In substrate, more than 50% of total As, Co, Mn and Ni are bio-accessible or potentially bio-
409 accessible, whereas less than 40% of total Fe, Zn and Mo are bio-accessible or potentially bio-
410 accessible. It is well reported that Co, Fe, Mn, Mo, Ni and Zn are important TE for optimal
411 performance of the anaerobic digestion processes as demonstrated by Gustavsson et al.
412 (Gustavsson et al., 2013, 2011) and reviewed by Zandvoort et al. (2006) and by Demirel and
413 Scherer (2011). Therefore, their bio-accessibility for anaerobic microorganisms is relevant. In
414 digestate, except for As, all elements have poor or limited bio-accessibility, suggesting the
415 formation of more stable forms of trace elements during anaerobic digestion process. This
416 information is relevant to fulfill national or European requirements for application of digestate as
417 soil amendment. Indeed, the current results show that less than 20% of total Cd, Cr, Cu, Ni, Pb
418 and Zn, considered harmful elements for plant uptake when present at high concentrations
419 (Saveyn and Eder, 2014), are immediately bio-accessible or potentially bio-accessible. However,
420 the other elements which serve as nutrient for plants also have poor or no bio-accessibility.

421 (Figure 3 here)

4. Conclusions

More than 60% of total As, Cd, Co, Fe, Mn, Ni and Zn were extracted during the fractionation procedure mainly in DOM and CSH fractions, which were defined as the immediately and poorly bio-accessible fractions, respectively. Between 31% and 98% of total elements was likely associated to minerals (CSH fraction) in both substrate and digestate, whereas the remaining elements were associated with OM. We observed that SEOM fraction mainly contains proteins, whereas PEOM contains hemicellulose, cellulose, starch and certain proteins. However, no specific organic molecules were extracted along with SPOM and REOM fractions.

Appendix A. Supplementary data

E-supplementary data for this work can be found in e-version of this paper online.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The Biogas Research Center at Linköping University is acknowledged for providing materials and laboratories to perform analytical analyses. Lena Lundman is also acknowledged for laboratory assistance and the Knut and Alice Wallenberg foundation program NMR for Life (www.nmrforlife.se) for NMR spectroscopy support. Finally, members of COST Action ES1302 European Network on Ecological Functions of Trace Metals in Anaerobic Biotechnologies are acknowledged for providing insightful comments to this experimental work.

Funding sources

This work was supported by the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 643071. This work was also supported by the Swedish Research Council Formas grant No 2016-01054.

Figure Captions

Figure 1. Analyses performed on the supernatants and solid residues collected during the sequential extraction procedure. DOC: dissolved organic carbon; TE: trace elements analysis; NMR: nuclear magnetic resonance spectroscopy; C/N: total carbon and nitrogen content; TS-VS: total and volatile solids content.

Figure 2. Organic matter characterization of substrate and digestate according to the sequential extraction procedure and NMR spectra. a-b) Relative distribution of organic C extracted after each step of the sequential extraction procedure form substrate and digestate samples. c-d)^{13}C CPMAS NMR spectra of DOM solid residues (n:3) from substrate and digestate. The integrated peak areas are expressed in % of total integral.

Figure 3. Interpretation of metals fractionation in terms of potential bio accessibility in substrate and digestate.

Table Captions

Table 1. Sequential extraction procedure adapted from Jimenez et al. (2017, 2014) with some modifications highlighted in italic font. The extracted fractions are listed in order of decreasing bio-accessibility.

Table 2. Target molecules extracted by the sequential extraction procedure adopted by Jimenez et al. (2017, 2015, 2014) and the one performed in this study. The nature of the organic molecules was identified by using reference samples and/or 3D fluorescence spectroscopy in the work of Jimenez et al. (2017, 2015, 2014), whereas NMR spectroscopy was used in this study.

465 **Table 3**. Trace elements concentration found in each extracted fraction and total elements
466 concentration in digestate (grey rows) and substrate (white rows). Except of DOM fraction
467 extraction, results are mean of triplicate ± standard deviation.

468

469

Tables and Figures

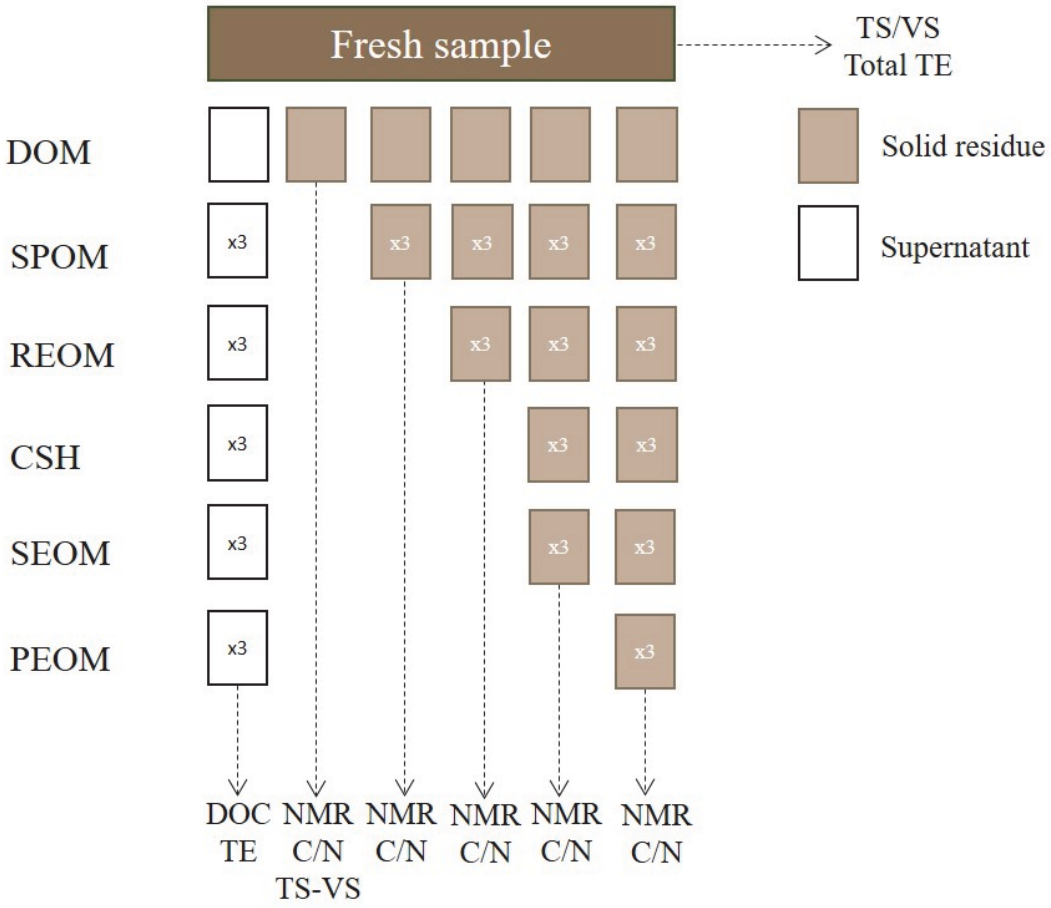
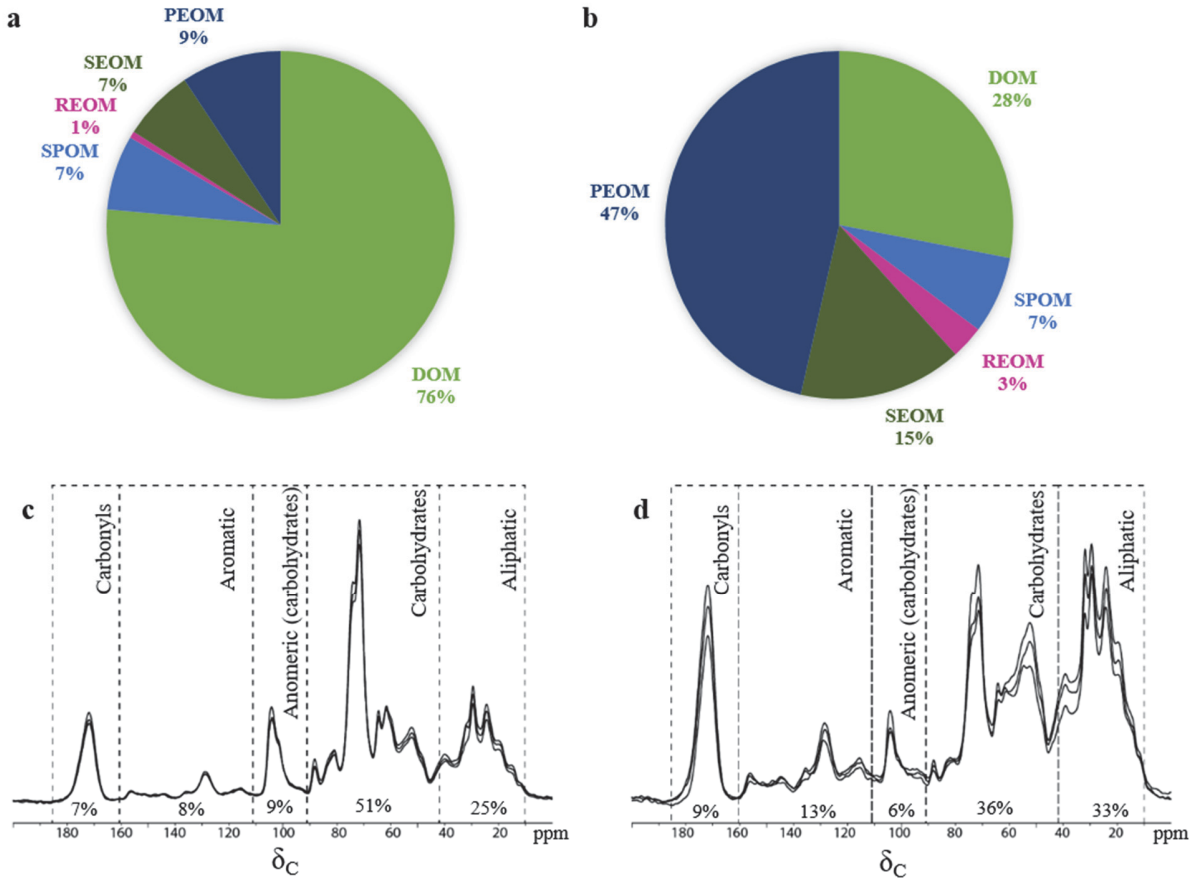
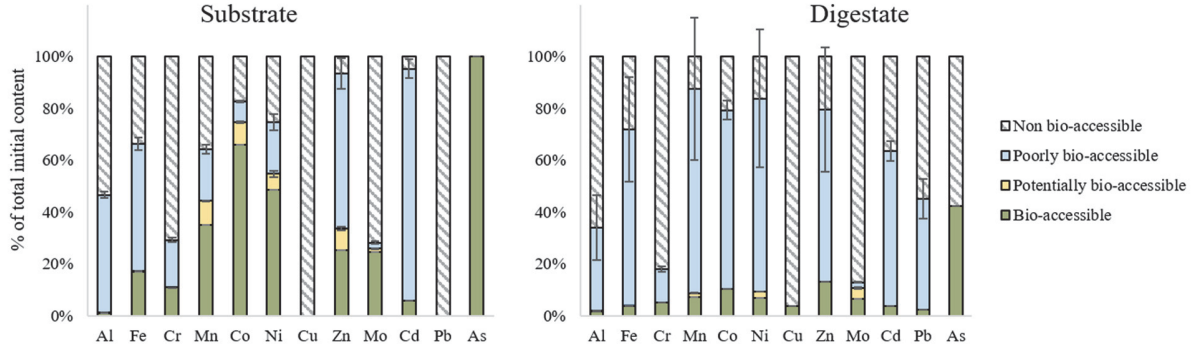


Figure 1.



473

474 **Figure 2.**



475

476 **Figure 3.**

477 **Table 1.**

OM Fraction	Reagent	Extraction Method	Bio-accessibility Degree
DOM	-	Centrifugation (18600g, 30 min, *10°C*), filtration 0.45 µm, N_2 *flushing*	High
SPOM	*24 ml* of 10 mM $CaCl_2$	4 × shaking (*200 rpm*, 30°C, 15 min), centrifugation, filtration, N_2 *flushing*	
REOM	*24 ml* of 10 mM NaCl + 10 mM NaOH	4 × shaking (200 rpm, 30°C, 15 min), centrifugation, filtration, N_2 *flushing*	
CSH	*24 ml* of 0.1 M HCl + ultrapure water rinsing	1 × shaking (200 rpm, 30°C, 60 min), centrifugation, filtration, N_2 *flushing*	
SEOM	*24 ml* of 0.1 M NaOH	4 × shaking (200 rpm, 30°C, 60 min), centrifugation, filtration, N_2 flushing	
PEOM	*24 ml* of 72% H_2SO_4	2 × shaking (*200 rpm*, 30°C, 3 h), centrifugation, filtration, N_2 *flushing*	Low

478

479

480 **Table 2.**

Fractions	Target molecules by Jimenez et al. (2017, 2015, 2014) (Reference samples + 3D fluorescence spectroscopy)	Target molecules according to this study (NMR spectroscopy)
SPOM	Water-soluble proteins and sugars	*The reagent did not selectively remove OM*
REOM	Proteins and lipids	*The reagent did not selectively remove OM*
SEOM	Humic-like and fulvic like acids, complex proteins (i.e. glucolated proteins) and certain lignocellulosic compounds	Certain proteins (CH(α) groups of the amino acids; methionine CH_3-groups; aromatic side-chains of amino acids)
PEOM	Hemicellulose and cellulose	Carbohydrate (e.g. hemicellulose, cellulose and starch) and certain proteins
NEOM	Lignin-like compounds and non-extractable humic-like acids (i.e. humin)	Protein/biomass (aliphatic and aromatic CH groups)

481

482 **Table 3.**

	DOM ($\mu g/gTS_{in}$)	SPOM ($\mu g/gTS_{in}$)	REOM ($\mu g/gTS_{in}$)	CSH ($\mu g/gTS_{in}$)	SEOM ($\mu g/gTS_{in}$)	Total ($\mu g/gTS_{in}$)	% of total content
Al	23.2	1.4±0.1	7.4±0.8	386.6±147.1	44.2±23.6	1359.4±33.7	34%
	7.7	0.9±0.2	<1.0#	318.0±8.6	10.3±1.0	722.1±59.7	47%
As	0.6	<0.1*	<0.02#	<0.4*	<0.1#	1.5±0.1	42%
	0.4	<0.1*	<0.01#	<0.2#	<0.1#	0.22±0.02	180%
Cd	0.01	<0.1#	<0.0003#	0.13±0.01	<0.004*	0.22±0.01	63%
	0.01	<0.04#	<0.01#	0.09±0.00	<0.001#	0.10±0.00	95%
Co	1.1	<0.2*	0.05±0.00	6.9±0.4	0.28±0.02	10.5±0.1	79%
	2.7	0.35±0.01	0.01±0.00	0.32±0.02	<0.01*	4.04±0.04	83%
Cr	0.3	<0.1#	<0.01#	0.7±0.1	0.08±0.00	5.9±0.2	18%
	0.2	<0.1#	<0.01*	0.32±0.01	0.05±0.00	2.0±0.1	29%
Cu	1.4	<0.9#	<0.8#	<3.4*	<0.1*	40.4±6.4	4%
	0.2	<0.5#	<0.5#	6.2±0.1	<0.4#	<35.8§	-
Fe	471.8	12.8±1.0	9.1±0.7	8502.1±2535.0	68.7±9.0	12623.1±222.8	72%
	749.3	6.8±2.1	2.9±0.3	2119.9±108.3	36.1±1.9	4393.0±71.0	66%
Mn	8.7	1.9±0.1	0.18±0.02	95.0±33.3	0.4±0.1	121.1±5.1	88%
	16.2	4.2±0.1	0.15±0.02	9.0±0.8	0.07±0.00	46.1±1.5	64%
Mo	0.2	0.09±0.01	0.04±0.00	0.01±0.00	<0.1#	2.3±0.1	13%
	0.2	0.01±0.00	0.02±0.00	<0.02*	<0.1#	0.68±0.02	28%
Ni	1.6	0.56±0.01	0.17±0.02	17.1±6.2	0.22±0.02	23.5±0.2	84%
	1.3	0.17±0.03	<0.01*	0.5±0.1	0.03±0.01	2.7±0.1	75%
Pb	0.1	<0.1#	<0.04#	1.6±0.3	<0.1*	3.8±0.6	45%
	0.01	<0.05#	<0.02#	0.6±0.1	<0.1*	<1.8§	-

Zn	22.3	<0.7#	<1.1#	109.8±40.3	2.0±0.1	168.4±6.8	80%
	17.2	5.6±0.5	<0.7#	39.3±3.7	1.5±0.3	68.1±0.6	93%

The percentage of total extracted is the ratio of the sum of element's concentration in DOM, SPOM, REOM, CSH and SEOM over the total element' concentration.

*MLQ=average blanks ± 10*standard deviation blanks (n=36) expressed on the same concentration basis (µg/gTS_{in}) as those for the samples using 25.6 gTS_{in}/l and 41.6 gTS_{in}/l as conversion factor for digestate and substrate respectively.

#MLD=average blanks ± 3*standard deviation blanks (n=36)

§MLQ=average blanks ± 10*standard deviation blanks (n=3), using 10.5 gTS_{in}/l as conversion factor.

483

References

1. Albacete, M.G., Tarquis, A.M., Cartagena, M.C., 2015. Risk of Leaching in Soils Amended by Compost and Digestate from Municipal Solid Waste 2014, 1–12. https://doi.org/10.1155/2014/565174
2. Aquino, S.F., Stuckey, D.C., 2007. Bioavailability and Toxicity of Metal Nutrients during Anaerobic Digestion. J. Environ. Eng. 133, 28–35. https://doi.org/10.1061/(ASCE)0733-9372(2007)133:1(28)
3. Bacon, J.R., Davidson, C.M., 2008. Is there a future for sequential chemical extraction? Analyst 133, 25–46. https://doi.org/10.1039/b711896a
4. Bajgiran, A.R., 2013. Influence of soil amendments and soil properties on macro–and micronutrient availability to microorganisms and plants. Swedish University of Agricultural Sciences, Uppsala, Sweden.
5. Braga, A.F.M., Zaiat, M., Silva, G.H.R., Fermoso, F.G., 2017. Metal fractionation in sludge from sewage UASB treatment. J. Environ. Manage. 193, 98–107. https://doi.org/10.1016/j.jenvman.2017.01.070
6. Callander, I.J., Barford, J.P., 1983. Precipitation, chelation, and the availability of metals as nutrients in anaerobic digestion. I. Methodology. Biotechnol. Bioeng. 25, 1947–1957. https://doi.org/10.1002/bit.260250805
7. Cao, W., Wang, M., Liu, M., Zhang, Z., Sun, Z., Miao, Y., Sun, C., Hu, C., 2018. The chemical and dynamic distribution characteristics of iron, cobalt and nickel in three different anaerobic digestates: effect of pH and trace elements dosage. Bioresour. Technol. 269, 363–374. https://doi.org/10.1016/j.biortech.2018.08.094
8. D'Abzac, P., Bordas, F., Joussein, E., Van Hullebusch, E., Lens, P.N.L., Guibaud, G.,

2010. Characterization of the mineral fraction associated to extracellular polymeric substances (EPS) in anaerobic granular sludges. Environ. Sci. Technol. 44, 412–418. https://doi.org/10.1021/es901912g

9. Demirel, B., Scherer, P., 2011. Trace element requirements of agricultural biogas digesters during biological conversion of renewable biomass to methane. Biomass and Bioenergy 35, 992–998. https://doi.org/10.1016/j.biombioe.2010.12.022
10. Feng, X.M., Karlsson, A., Svensson, B.H., Bertilsson, S., 2010. Impact of trace element addition on biogas production from food industrial waste--linking process to microbial communities. FEMS Microbiol. Ecol. 74, 226–40. https://doi.org/10.1111/j.1574-6941.2010.00932.x
11. Fermoso, F.G., van Hullebusch, E.D., Guibaud, G., Collins, G., Svensson, B.H., Carliell-Marquet, C., Vink, J.P.M., Esposito, G., Frunzo, L., 2015. Fate of Trace Metals in Anaerobic Digestion, in: Guebitz, G.M., Bauer, A., Bochmann, G., Gronauer, A., Weiss, S. (Eds.), Biogas Science and Technology. Springer International Publishing, Switzerland, pp. 171–195. https://doi.org/10.1007/978-3-319-21993-6_7
12. Filgueiras, A. V., Lavilla, I., Bendicho, C., 2002. Chemical sequential extraction for metal partitioning in environmental solid samples. J. Environ. Monit. 4, 823–857. https://doi.org/10.1039/b207574c
13. Gonzalez-Gil, G., Jansen, S., Zandvoort, M.H., van Leeuwen, H.P., 2003. Effect of yeast extract on speciation and bioavailability of nickel and cobalt in anaerobic bioreactors. Biotechnol. Bioeng. 82, 134–42. https://doi.org/10.1002/bit.10551
14. Gustavsson, J., Shakeri Yekta, S., Sundberg, C., Karlsson, A., Ejlertsson, J., Skyllberg, U., Svensson, B.H., 2013. Bioavailability of cobalt and nickel during anaerobic digestion of sulfur-rich stillage for biogas formation. Appl. Energy 112, 473–477.

https://doi.org/10.1016/j.apenergy.2013.02.009

15. Gustavsson, J., Svensson, B.H., Karlsson, A., 2011. The feasibility of trace element supplementation for stable operation of wheat stillage-fed biogas tank reactors. Water Sci. Technol. 64, 320–325. https://doi.org/10.2166/wst.2011.633
16. Harmsen, J., 2007. Measuring Bioavailability: From a Scientific Approach to Standard Methods. J. Environ. Qual. 36, 1420. https://doi.org/10.2134/jeq2006.0492
17. Houba, V.J.G., Lexmond, T.M., Novozamsky, I., Van Der Lee, J.J., 1996. State of the art and future developments in soil analysis for bioavailability assessment. Sci. Total Environ. 178, 21–28. https://doi.org/10.1016/0048-9697(95)04793-X
18. Jimenez, J., Aemig, Q., Doussiet, N., Steyer, J.P., Houot, S., Patureau, D., 2015. A new organic matter fractionation methodology for organic wastes: Bioaccessibility and complexity characterization for treatment optimization. Bioresour. Technol. 194, 344–353. https://doi.org/10.1016/j.biortech.2015.07.037
19. Jimenez, J., Gonidec, E., Cacho Rivero, J.A., Latrille, E., Vedrenne, F., Steyer, J.-P., 2014. Prediction of anaerobic biodegradability and bioaccessibility of municipal sludge by coupling sequential extractions with fluorescence spectroscopy: Towards ADM1 variables characterization. Water Res. 50, 359–372. https://doi.org/10.1016/j.watres.2013.10.048
20. Jimenez, J., Lei, H., Steyer, J.P., Houot, S., Patureau, D., 2017. Methane production and fertilizing value of organic waste: Organic matter characterization for a better prediction of valorization pathways. Bioresour. Technol. 241, 1012–1021. https://doi.org/10.1016/j.biortech.2017.05.176
21. Karlsson, A., Einarsson, P., Schnürer, A., Sundberg, C., Ejlertsson, J., Svensson, B.H., 2012. Impact of trace element addition on degradation efficiency of volatile fatty acids,

554 oleic acid and phenyl acetate and on microbial populations in a biogas digester. J. Biosci.
555 Bioeng. 114, 446–52. https://doi.org/10.1016/j.jbiosc.2012.05.010
556 22. Keeler, C., Kelly, E.F., Maciel, G.E., 2006. Chemical-structural information from solid-
557 state 13C NMR studies of a suite of humic materials from a lower montane forest soil,
558 Colorado, USA. Geoderma 130, 124–140.
559 https://doi.org/10.1016/j.geoderma.2005.01.015
560 23. Knoop, C., Tietze, M., Dornack, C., Raab, T., 2018. Fate of nutrients and heavy metals
561 during two-stage digestion and aerobic post-treatment of municipal organic waste.
562 Bioresour. Technol. 251, 238–248. https://doi.org/10.1016/j.biortech.2017.12.019
563 24. Kögel-Knabner, I., 1997. 13C and 15N NMR spectroscopy as a tool in soil organic matter
564 studies. Geoderma 80, 243–270. https://doi.org/10.1016/S0016-7061(97)00055-4
565 25. Kupper, T., Bürge, D., Bachmann, H.J., Güsewell, S., Mayer, J., 2014. Heavy metals in
566 source-separated compost and digestates. Waste Manag. 34, 867–874.
567 https://doi.org/10.1016/j.wasman.2014.02.007
568 26. Lindorfer, H., Ramhold, D., Frauz, B., 2012. Nutrient and trace element supply in
569 anaerobic digestion plants and effect of trace element application. Water Sci. Technol. 66,
570 1923–1929. https://doi.org/10.2166/wst.2012.399
571 27. Maharaj, B.C., Mattei, M.R., Frunzo, L., Hullebusch, E.D. va., Esposito, G., 2019. ADM1
572 based mathematical model of trace element complexation in anaerobic digestion
573 processes. Bioresour. Technol. 276, 253–259.
574 https://doi.org/10.1016/j.biortech.2018.12.064
575 28. Maharaj, B.C., Mattei, M.R., Frunzo, L., van Hullebusch, E.D., Esposito, G., 2018.
576 ADM1 based mathematical model of trace element precipitation/dissolution in anaerobic
577 digestion processes. Bioresour. Technol. 267, 666–676.

https://doi.org/10.1016/j.biortech.2018.06.099

29. Nkoa, R., 2014. Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: A review. Agron. Sustain. Dev. 34, 473–492. https://doi.org/10.1007/s13593-013-0196-z

30. Ortner, M., Rachbauer, L., Somitsch, W., Fuchs, W., 2014. Can bioavailability of trace nutrients be measured in anaerobic digestion? Appl. Energy 126, 190–198. https://doi.org/10.1016/j.apenergy.2014.03.070

31. Rauret, G., Lorez-Sanchez, J.F., Sahuquillo, A., Rubio, C., Ure, A., Quevauviller, P., 1991. Improvement of BCR three step sequential extraction procedure to the certification of new sediment and soil reference materials. Enviroment Monit. 1, 57–61.

32. Rickard, D., Morse, J.W., 2005. Acid volatile sulfide (AVS), Marine Chemistry. https://doi.org/10.1016/j.marchem.2005.08.004

33. Saveyn, H., Eder, P., 2014. End-of-waste criteria for biodegradable waste subjected to biological treatment (compost & digestate): Technical proposals. https://doi.org/10.2791/6295

34. Shakeri Yekta, S., Hedenström, M., Stehr, J.E., Dario, M., Hertkorn, N., Björn, A., 2018. Pretreatment of anaerobic digester samples by hydrochloric acid for solution-state 1H and 13C NMR spectroscopic characterization of organic matter. Chemosphere 199, 201–209. https://doi.org/10.1016/j.chemosphere.2018.02.015

35. Shakeri Yekta, S., Lindmark, A., Skyllberg, U., Danielsson, Å., Svensson, B.H., 2014a. Importance of reduced sulfur for the equilibrium chemistry and kinetics of Fe(II), Co(II) and Ni(II) supplemented to semi-continuous stirred tank biogas reactors fed with stillage. J. Hazard. Mater. 269, 83–88. https://doi.org/10.1016/j.jhazmat.2014.01.051

36. Shakeri Yekta, S., Svensson, B.H., Björn, A., Skyllberg, U., 2014b. Thermodynamic

602 modeling of iron and trace metal solubility and speciation under sulfidic and ferruginous
603 conditions in full scale continuous stirred tank biogas reactors. Appl. Geochemistry 47,
604 61–73. https://doi.org/10.1016/j.apgeochem.2014.05.001
605 37. Simpson, A.J., McNally, D.J., Simpson, M.J., 2011. NMR spectroscopy in environmental
606 research: From molecular interactions to global processes. Prog. Nucl. Magn. Reson.
607 Spectrosc. 58, 97–175. https://doi.org/10.1016/j.pnmrs.2010.09.001
608 38. Soucémarianadin, L.N., Erhagen, B., Nilsson, M.B., Öquist, M.G., Immerzeel, P.,
609 Schleucher, J., 2017. Two dimensional NMR spectroscopy for molecular characterization
610 of soil organic matter: Application to boreal soils and litter. Org. Geochem. 113, 184–195.
611 https://doi.org/10.1016/j.orggeochem.2017.06.019
612 39. Tambone, F., Adani, F., Gigliotti, G., Volpe, D., Fabbri, C., Provenzano, M.R., 2013.
613 Organic matter characterization during the anaerobic digestion of different biomasses by
614 means of CPMAS ^{13}C NMR spectroscopy. Biomass and Bioenergy 48, 111–120.
615 https://doi.org/10.1016/j.biombioe.2012.11.006
616 40. Tambone, F., Genevini, P., D'Imporzano, G., Adani, F., 2009. Assessing amendment
617 properties of digestate by studying the organic matter composition and the degree of
618 biological stability during the anaerobic digestion of the organic fraction of MSW.
619 Bioresour. Technol. 100, 3140–3142. https://doi.org/10.1016/j.biortech.2009.02.012
620 41. Thanh, P.M., Ketheesan, B., Yan, Z., Stuckey, D., 2016. Trace metal speciation and
621 bioavailability in anaerobic digestion: A review. Biotechnol. Adv. 34, 122–136.
622 https://doi.org/10.1016/j.biotechadv.2015.12.006
623 42. van Hullebusch, E.D., Utomo, S., Zandvoort, M.H., Lens, P.N.L.L., 2005. Comparison of
624 three sequential extraction procedures to describe metal fractionation in anaerobic
625 granular sludges. Talanta 65, 549–558. https://doi.org/10.1016/j.talanta.2004.07.024

626 43. van Reeuwijk, L.P., Houba, V.J.G., 1998. Chapter 7 Quality of Analytical Procedures
627 [WWW Document]. Guidel. Qual. Manag. Soil Plant Lab. (FAO Soils Bull. - 74). URL
628 http://www.fao.org/docrep/W7295E/w7295e09.htm#7.5.1 trueness (accuracy), bias
629 (accessed 4.2.17).
630 44. Zandvoort, M.H., van Hullebusch, E.D., Fermoso, F.G., Lens, P.N.L., 2006. Trace Metals
631 in Anaerobic Granular Sludge Reactors: Bioavailability and Dosing Strategies. Eng. Life
632 Sci. 6, 293–301. https://doi.org/10.1002/elsc.200620129
633 45. Zhu, N., Li, Q., Guo, X., Zhang, H., Deng, Y., 2014. Sequential extraction of anaerobic
634 digestate sludge for the determination of partitioning of heavy metals. Ecotoxicol.
635 Environ. Saf. 102, 18–24. https://doi.org/10.1016/j.ecoenv.2013.12.033
636 46. Zufiaurre, R., Olivar, A., Chamorro, P., Nerin, C., Callizo, A., 1998. Speciation of metals
637 in sewage sludge for agricultural uses. Analyst 123, 255–259.
638 https://doi.org/10.1039/a705168i

639

Supplementary data for

A simultaneous assessment of organic matter and trace elements bio-accessibility in substrate and digestate from an anaerobic digestion plant

Authors and affiliation

Andreina Laera[a,d,*], Sepehr Shakeri Yekta[b], Mattias Hedenström[c], Rémy Buzier[d], Gilles Guibaud[d], Mårten Dario[b], Giovanni Esposito[e], Eric D. van Hullebusch[f]

[a] University of Paris-Est, Laboratoire Géomatériaux et Environnement (EA 4508), UPEM, 77454 Marne-la-Vallée, France, andreina.laera@u-pem.fr or piedelibero89@gmail.com

[b] Department of Thematic Studies-Environmental Change and Biogas Research Center, Linköping University, 581 83 Linköping, Sweden

[c] Department of Chemistry, Umeå University, 901 87 Umeå, Sweden

[d] University of Limoges, PEIRENE, Equipe Développement d'indicateurs ou prévision de la qualité des eaux, URA IRSTEA, 123 Avenue Albert Thomas, 87060 Limoges Cedex, France

[e] University of Napoli "Federico II", Department of Civil, Architectural and Environmental Engineering, via Claudio 21, 80125 Napoli, Italy

[f] Université de Paris, Institut de Physique du Globe de Paris, CNRS, UMR 7154, F-75238 Paris, France

*Corresponding author: andreina.laera@u-pem.fr

Table of content

Matrix effect

The method adopted to evaluate matrix effect consists of calculating the slope of a standard addition curve where the y-axis is the observed concentration of trace elements or organic carbon and the x-axis is the theoretical added concentration. The absence of matrix effect is identified when the slope of the curve is equal to 1. The standard addition curve was built for each reagent used during the sequential extraction procedure.

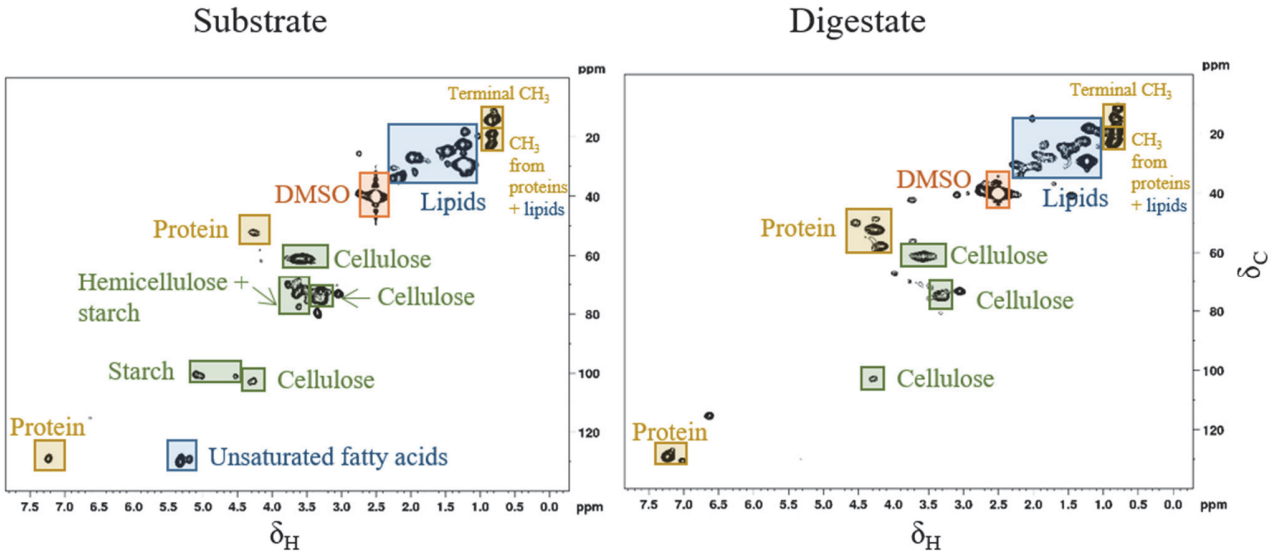


Figure 1. 2D ^{1}H-^{13}C HSQC NMR spectrum of DOM solid residue from substrate and digestate. The x and y-axis reports the chemical shift of ^{1}H and ^{13}C respectively in part per million (ppm) and peak originating from proteins, lipids and carbohydrates are marked in orange, blue and green, respectively.

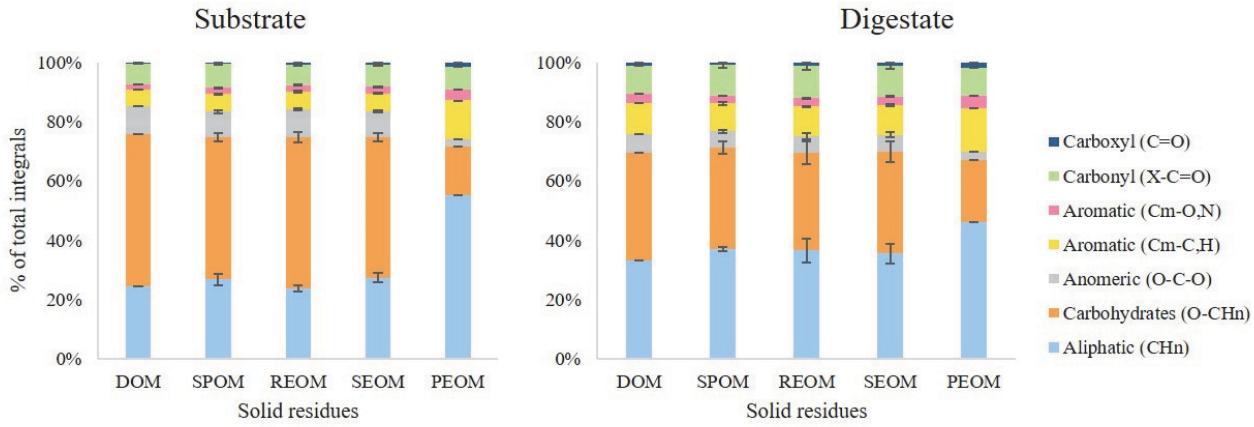
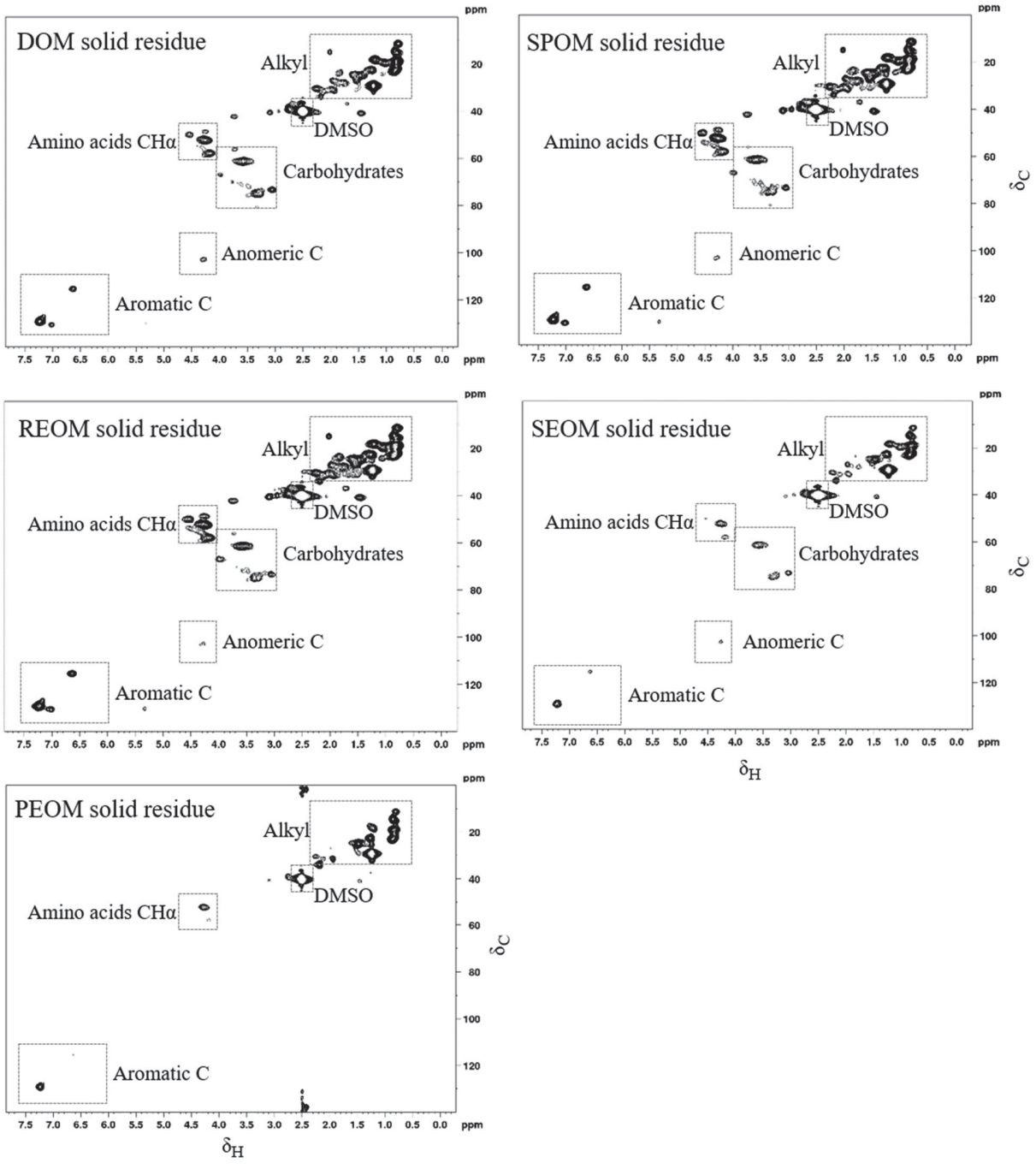


Figure 2. Distribution of C among different organic groups for the substrate and digestate solid residues. Results are expressed as % of total integrals of ^{13}C CPMAS NMR spectra.

Digestate


DOM solid residue
Alkyl
DMSO
Amino acids CHα
Carbohydrates
Anomeric C
Aromatic C
SPOM solid residue
REOM solid residue
SEOM solid residue
PEOM solid residue
ppm
δC
δH

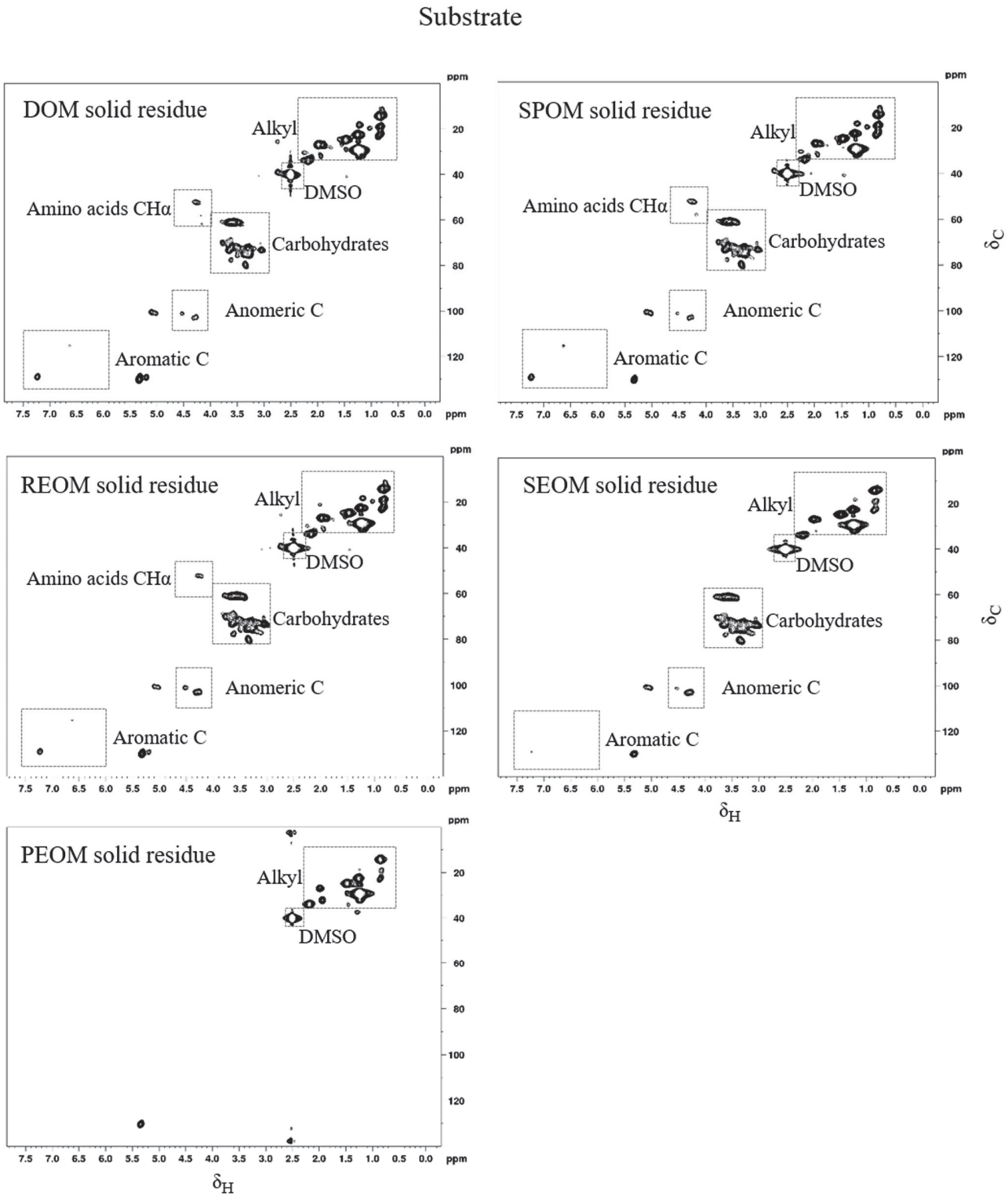


Figure 3. 2D ^{1}H-^{13}C HSQC NMR spectra of digestate and substrate solid residues collected during the sequential extraction procedure. Spectral regions representative for different chemical structures or functionalities are indicated by rectangles (Simpson et al., 2011; Soucémarianadin et al., 2017).

Table 1. Mass balance of C. Extracted dissolved organic C and initial C content are compared to estimate the percentage of non- and extracted C.

	Substrate	Digestate
Extracted dissolved organic C* (mg)	213.6	121.4
Initial C content[#] (mg)	546.0	316.9
Extracted C (%)	39.1	38.3
Non-extracted C (%)	60.9	61.7

*It is the sum of dissolved organic C extracted in SPOM, REOM, SEOM and PEOM fractions

[#] It is quantified in the solid residue after DOM extraction

Table 2. Trace elements concentration found in each extracted fraction by the modified sequential extraction procedure and total elements concentration in digestate (grey rows) and substrate (white rows). Except of DOM fraction extraction, results are mean of triplicate ± standard deviation

	DOM ($\mu g/gTS_{in}$)	**CSH** ($\mu g/gTS_{in}$)	SPOM ($\mu g/gTS_{in}$)	REOM ($\mu g/gTS_{in}$)	SEOM ($\mu g/gTS_{in}$)	Total ($\mu g/gTS_{in}$)	% of total content
Al	23.2	503.2±47.9	8.5±1.8	<1.7#	27.2±4.9	1359.4±33.7	41%
	7.7	403.5±33.9	7.9±1.8	<1.0#	10.3±0.1	722.1±59.7	59%
As	0.6	<0.3#	0.3±0.1	<0.02#	<0.1#	1.5±0.1	64%
	0.4	<0.2#	0.09±0.02	<0.01#	<0.1#	0.22±0.02	220%
Cd	0.01	0.14±0.02	<0.1#	<0.0003#	<0.001#	0.22±0.01	64%
	0.01	0.13±0.01	<0.04#	0.01±0.00	<0.001#	0.10±0.01	138%
Co	1.1	7.6±0.9	0.4±0.1	0.05±0.01	0.23±0.03	10.5±0.1	89%
	2.7	1.4±0.1	0.5±0.1	<0.01*	<0.01*	4.04±0.04	111%
Cr	0.3	0.67±0.05	0.04±0.00	<0.01#	0.10±0.00	5.9±0.2	19%
	0.2	0.36±0.03	<0.1#	<0.01*	0.06±0.00	2.0±0.1	32%
Cu	1.4	<1.5#	<3.2#	<0.8#	<1.8*	40.4±6.4	4%
	0.2	6.4±0.3	<0.5#	<0.5#	<0.4#	<35.8§	-
Fe	471.8	11322.4±2116.7	234.4±59.8	21.0±10.2	50.3±4.8	12623.1±222.8	96%
	749.3	2390.1±133.3	44.2±4.8	4.7±1.6	47.0±1.4	4393.0±71.0	74%
Mn	8.7	124.6±10.4	2.3±0.8	0.1±0.1	<0.1*	121.1±5.1	112%
	16.2	20.3±2.1	5.4±1.5	0.2±0.1	<0.04*	46.1±1.5	91%
Mo	0.2	<0.04*	<0.1#	<0.01*	<0.1#	2.3±0.1	7%
	0.2	<0.02*	<0.01#	0.01±0.00	<0.1#	0.68±0.02	26%
Ni	1.6	22.1±2.7	2.9±0.5	0.08±0.04	0.15±0.03	23.5±0.2	114%
	1.3	0.9±0.1	0.3±0.1	<0.01#	<0.02*	2.7±0.1	95%
Pb	0.1	1.3±0.5	<0.1#	<0.03#	<0.1*	3.8±0.6	37%
	0.0	0.46±0.03	<0.04#	<0.02#	<0.1*	<1.8§	-

Zn	22.3	140.2±10.3	4.6±1.7	<1.1#	<1.4*	168.4±6.8	99%
	17.2	51.6±2.8	4.0±1.2	<0.7#	1.3±0.5	68.1±0.6	109%

*MLQ=average blanks ± 10*standard deviation blanks (n=36) expressed on the same concentration basis (µg/gTS_{in}) as those for the samples using 25.6 gTS_{in}/l and 41.6 gTS_{in}/l as conversion factor for digestate and substrate respectively.

#MLD=average blanks ± 3*standard deviation blanks (n=36)

§MLQ=average blanks ± 10*standard deviation blanks (n=3), using 10.5 gTS_{in}/l as conversion factor.

References

Simpson, A.J., McNally, D.J., Simpson, M.J., 2011. NMR spectroscopy in environmental research: From molecular interactions to global processes. Prog. Nucl. Magn. Reson. Spectrosc. 58, 97–175. https://doi.org/10.1016/j.pnmrs.2010.09.001

Soucémarianadin, L.N., Erhagen, B., Nilsson, M.B., Öquist, M.G., Immerzeel, P., Schleucher, J., 2017. Two dimensional NMR spectroscopy for molecular characterization of soil organic matter: Application to boreal soils and litter. Org. Geochem. 113, 184–195. https://doi.org/10.1016/j.orggeochem.2017.06.019

II

ASSESSMENT OF THE DGT TECHNIQUE IN DIGESTATE TO FRACTION TWELVE TRACE ELEMENTS

by

Andreina Laera, Rémy Buzier, Gilles Guibaud, Giovanni Esposito & Eric D. van Hullebusch, 15 January 2019

Talanta 192, 204-211

Reproduced with kind permission by Elsevier.

Talanta 192 (2019) 204–211

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Assessment of the DGT technique in digestate to fraction twelve trace elements

Andreina Laera[a,b,c], Rémy Buzier[b,*], Gilles Guibaud[b], Giovanni Esposito[c], Eric D. van Hullebusch[a,d]

[a] University of Paris-Est, Laboratoire Géomatériaux et Environnement (EA 4508), UPEM, 77454 Marne-la-Vallée, France
[b] University of Limoges, PEIRENE, Equipe Développement d'indicateurs ou prévision de la qualité des eaux, URA IRSTEA, 123 Avenue Albert Thomas, 87060 Limoges Cedex, France
[c] University of Cassino and Southern Lazio, Department of Civil and Mechanical Engineering, via Gaetano di Biasio 43, 03043 Cassino (FR), Italy
[d] IHE Delft Institute for Water Education, Department of Environmental Engineering and Water Technology, PO Box 3015, 2601 DA Delft, The Netherlands

ARTICLE INFO

Keywords:
Digested sewage sludge
Passive sampling
Matrix interferences
Metals
Metalloids
Speciation

ABSTRACT

This study proposes an evaluation of the diffusive gradients in thin films technique (DGT) for studying trace elements in digested sewage sludge samples. Twelve elements were monitored by Chelex (Al, Cd, Co, Cr (III), Cu, Fe, Mn, Ni, Pb) and zirconia-DGT (As, Mo, Se) samplers exposed from 4 h to 9 days. Twenty-four hours' deployment time was suitable for most of the studied elements. However, short deployment led to insufficient element accumulation or non-establishment of steady state while long deployment (from 18 to 144 h depending on the element) led to saturation of the binding gels and/or competing effects with other major elements. In addition, this study showed that the matrix of the digested sewage sludge lowers the accumulation of some trace elements in the DGT samplers, leading to labile concentrations underestimation of roughly 10–30% (depending on the element). Moreover, compared to the conventional total dissolved elements measurement, DGT technique allowed to quantify 7 out of 12 labile elements whereas only 3 out of 12 dissolved elements were quantified. These results highlight the potential of DGT technique to assess labile trace elements in digestate samples, provided a careful adaptation of the deployment time as well as an evaluation of the matrix effect is performed.

1. Introduction

Knowledge regarding trace elements' speciation is fundamental to assess their bio-accessibility in digestate. Given the complexity of the matrix and wide diversity of metal species (*e.g.* complexes, precipitates…) encountered in digestate, fractionation approaches are commonly used for metal speciation purposes [1].

Few studies [2–4] attempted to fractionate trace elements in digested sewage sludge by chemical sequential extraction procedures to determine the degree of leachability of different trace elements' species. Zhu et al. [4] underlined that sequential extraction methods could be used for environmental risk assessment of digestate as a soil fertilizer. However, Bacon and Davidson [5] have questioned the usefulness of sequential extraction procedures to fractionate trace elements. The authors highlighted some limitations in quantifying trace elements associated with several mineral phases extracted during these procedures. Such limitations include the re-distribution of the element among the mineral phases and precipitation during the extraction, the non-selectivity of the reagents to the targeted phases and their incomplete extraction [5].

To overcome the limitations of sequential extraction procedures, in a recent paper, Thanh et al. [6] identified the diffusive gradients in thin films technique (DGT) as a promising technique to determine bio-accessible metal concentrations in anaerobic bioreactors. This technique allows sampling labile trace elements after diffusion through a gel and accumulation on a binding gel in the DGT device [7]. The labile elements comprise free ions and weakly bound complexes and thereby would represent the most readily bio-accessible species of trace elements [7]. Recently, Bourven et al. [8] demonstrated a link between DGT-labile Cd concentrations and biogas production as well as enzymatic activities during whey anaerobic digestion. However, DGT use in digestate is emerging and, to our knowledge, only Takashima et al. [9] has used the DGT technique to measure labile Co and Ni species in a digested sewage sludge filtrate. Currently, no methodological development has been performed to adapt this technique to the digestate matrix. Moreover, the use of DGT is not straightforward in such

* Corresponding author.
E-mail addresses: andreina.laera@u-pem.fr (A. Laera), remy.buzier@unilim.fr (R. Buzier).

https://doi.org/10.1016/j.talanta.2018.09.033
Received 5 June 2018; Received in revised form 31 August 2018; Accepted 11 September 2018
Available online 12 September 2018
0039-9140/ © 2018 Elsevier B.V. All rights reserved.

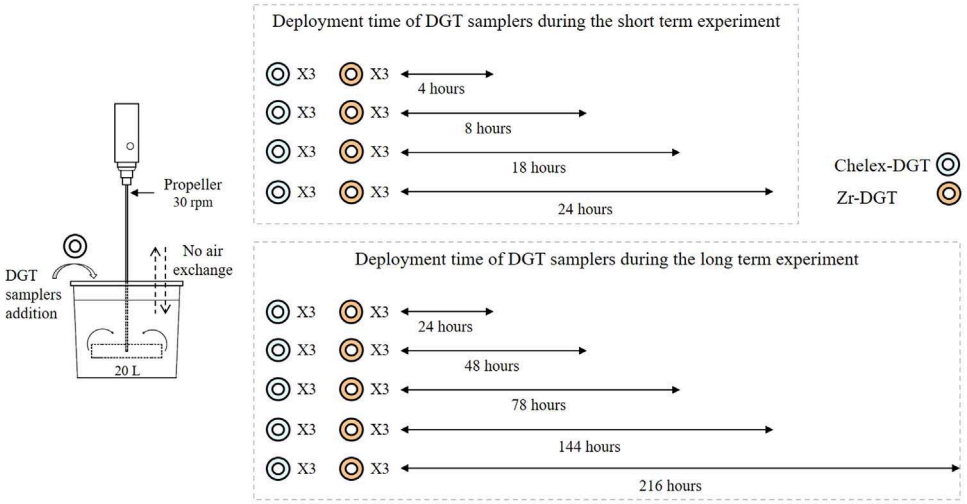


Fig. 1. On the left, the pilot scale tank containing the digested sludge. On the right, a scheme of the deployment time of the Chelex and Zr-DGT samplers.

complex matrix (*e.g.* multi-element contamination, high organic content) and requires preliminary validation or adaptation of the procedure.

We sought to investigate the potential of DGT as a fractionation tool for twelve trace elements (Al, As, Cd, Co, Cr (III), Cu, Fe, Mn, Mo, Ni, Pb and Se) in anaerobic digestate. Experiments were performed to validate the principles of the method in this complex biological matrix and to investigate potential organic matter interferences on trace elements' accumulation in DGT devices. Moreover, to discriminate large labile complexes from small ones, we performed fractionation based on the size of trace elements by using two different diffusive layers in our DGT devices.

The outcomes of this research work will highlight the benefits and limitations of using the DGT tool to assess labile trace elements in digestate samples and we offer recommendations to help establishing robust DGT deployment methods in digestates.

2. Materials and methods

2.1. Digested sewage sludge sample

Digested sewage sludge was collected from a municipal waste-water treatment plant in Limoges, France. About 20 L of sample was collected in June and September 2017. The sample was collected in polypropylene (PP) tanks up to maximum capacity and closed with a lid to limit sample oxidation from dioxygen in the air. Later, they were stored at 4 °C for less than 24 h before starting the experiments.

2.2. DGT preparation

Two different DGT samplers were used during this study: Chelex-DGTs for cationic species (Al, Cd, Co, Cr (III), Cu, Fe, Mn, Ni and Pb) and zirconia-DGTs (Zr-DGTs) for anionic species (As, Mo and Se). The selectivity of Chelex-DGT sampler over the oxidation state of Cr species was previously demonstrated by Ernstberger et al. [10]. Each DGT consisted of a binding gel, a diffusive gel and a filter membrane enclosed in a piston type holder, the latter purchased from DGT Research (Lancaster, UK). Chelex binding gels were prepared according to the procedure described by Zhang et al. [11], whereas Zr binding gels were made according to Devillers et al. [12].

Unless stated otherwise, the DGT samplers were equipped with a standard polyacrylamide diffusive gel (15% acrylamide and 0.3% agarose-derived cross linker, 0.77 mm thick), prepared according to Zhang et al. [11]. In addition, the use of restricted diffusive gels (15% acrylamide and 0.75% bisacrylamide cross linker, 0.75 mm thick) with pore size < 1 nm [13] was investigated. The gels were prepared following a procedure slightly modified from Scally et al. [14]. The polymerization was performed by mixing 200 µL of 10% (m/V) freshly prepared ammonium persulfate (Fisher Scientific) and 8 µL of tetramethylethylenediamine (TEMED) (Aldrich) with 10 mL of gel solution (15% acrylamide and 0.75% bisacrylamide cross linker). The full procedure is described in Supporting information.

Protective membranes of 0.4 µm pore size Nuclepore® in polycarbonate (0.02 mm thickness, Whatman, UK) or 0.2 µm pore size cellulose acetate membrane (0.12 mm thickness, Whatman, UK) was placed on the top of the diffusive gel.

2.3. Experimental set-up

2.3.1. Optimization of DGT samplers' deployment time

About 20 L of digested sludge was poured into a PP container and continuously stirred with an overhead plastic propeller at 30 rpm. A Tinytag data logger (TG-4100, Gemini Data Loggers, UK) was used to record the temperature in the sample. To avoid changes of trace elements speciation, the sample was kept in anaerobic conditions by covering its surface with paraffin oil and a plastic film.

Two different experiments were performed: a "short term" one to validate the establishment of steady state conditions in the samplers, and a "long term" one to increase the sensitivity of the method. In detail, triplicate devices of both Chelex and Zr were deployed for 4, 8, 18 and 24 h ("short term" experiment) or for 24, 48, 72, 144 and 216 h (long term experiment). A representation of the experimental set-up is shown in Fig. 1. Before starting the experiment, the devices were immersed overnight in nitrogen flushed ultrapure water to remove oxygen from them.

2.3.2. Potential interference from digestate matrix on trace elements accumulation

To evaluate the potential interference from the digestate matrix on the diffusion and accumulation of trace elements in the binding gels, the Chelex and Zr-DGT samplers were exposed in triplicate to the digestate sample for 24 h to load their diffusive gels with the digestate matrix. The pre-exposed diffusive gels were then recovered to build new DGT samplers with new Chelex and Zr binding gels (henceforth named "soiled" DGT samplers). Additionally, triplicate DGT samplers were built with new diffusive and binding gels as control in the experiment.

All Chelex-DGT samplers (control and soiled) were immersed in 1.5 L of 10^{-2} M NaCl solution spiked with cationic elements (Cd (II), Co (II), Cu (II), Ni (II) and Pb (II)) for 4 h under continuous stirring. Al (III),

Cr (III), Fe (II) and Mn (II) were not added in the synthetic solution since they tend to precipitate. The control and "soiled" Zr-DGT samplers were deployed for 4 h under continuous stirring in a second beaker, containing 1.5 L of 10^{-2} M NaCl spiked with anionic elements (As (III), Mo (VI) and Se (IV)) and flushed with N_2 to avoid oxidation of the elements. The total concentrations of the elements were chosen to be either quantifiable or comparable to the studied digestate samples. The conditions of the experiments (pH, temperature and element concentration) are summarized in Tables S1 and S2.

To check the contamination of the binding gel brought by the "soiled" diffusive gel, three blank DGT samplers were built with "soiled" diffusive gels and new Chelex and Zr binding gels. The blanks were stored at room temperature (20 ± 1 °C) in a moistened plastic bag and disassembled after 4 h alongside the other samplers.

For statistical analysis of the results, a F-test was performed using Microsoft Excel 2013 to determine the variances of the two sets of samples, then the two-tailed *t*-test was applied at 95% confidence interval.

2.3.3. Size fractionation of labile elements

Fractionation of labile elements based on their size was investigated through the simultaneous deployment of DGT samplers equipped with restricted or standard diffusive gels. The Chelex and Zr-DGT samplers were deployed for 24 h in 20 L of digested sludge sample continuously stirred at 30 rpm. The deployment time was chosen according to the results obtained from the experiment described in 2.3.1.

2.4. Analytical procedures

2.4.1. DGT-labile concentration

After retrieval, DGT samplers were rinsed with ultrapure water and disassembled to recover the binding gels. The accumulated mass (m) of trace elements in each DGT sampler was determined after elution of the binding gel. The Chelex binding gels were eluted in 2 mL of 1 M HNO_3 for 24 h and the Zr binding gels in 2 mL of $5{\cdot}10^{-3}$ M NaOH and 0.5 M H_2O_2 for 24 h. Then the concentration of trace elements in the eluents (C_e) were quantified by the inductively coupled plasma mass spectrometry (ICP-MS) or microwave plasma atomic emission spectroscopy (MP-AES) (see section 2.4.3). The accumulated mass is determined according to Eq. (1) [15]:

$$m = \frac{C_e \times V_e}{f_e}, \quad (1)$$

where V_e is the volume of the eluents (2 mL) and f_e is the elution factor (values are reported in Table S3).

The concentration of labile trace elements, C_{DGT}, in the sample is then derived using Eq. (2) based on Fick's first law [16]:

$$C_{DGT} = \frac{m \times \Delta_{MDL}}{D \times t \times A}, \quad (2)$$

where Δ_{MDL} is the thickness of the material diffusion layer (*i.e.* diffusive gel plus membrane), t is the time of DGT samplers' exposure in the sludge, D is the coefficient of diffusion of the considered element in the diffusion layer and A is the geometric area of the DGT holder window (3.14 cm^2). The values of D were corrected for the average temperature (T) recorded every 10 min by a Tinytag data logger during each deployment using Stokes–Einstein relation [13] as follows:

$$\frac{D_1 \times \eta_1}{T_1} = \frac{D_2 \times \eta_2}{T_2}, \quad (3)$$

where η is the viscosity of the water taken from the NIST chemistry WebBook [17]. The values of D at 25 °C used in our study for a standard diffusive gel are summarized in Table S4 in supporting information. The D values for the restricted gel are equal to 70% of the D for a standard gel, based on the work of Scally et al. [14] and Shiva et al. [18] as summarized in Table S5.

2.4.2. Physicochemical analysis

The pH was measured with a Mettler Toledo pH electrode. The total solids (TS), volatile solids (VS), total suspended solids (TSS) and volatile suspended solids (VSS) were measured according to the French standard AFNOR NF T90–105 method. The supernatant recovered during the TSS and VSS procedure was used to estimate dissolved trace elements (see section 2.4.3).

2.4.3. Sample treatment and trace elements analysis

At the beginning and at the end of each experiment, an aliquot of digested sewage sludge was sampled to measure the total and dissolved elements' content. About 5 g of raw sample (total content) or 2 mL of supernatant (dissolved content), recovered after centrifugation at 3.000 g for 20 min, were digested with 6 mL of 69% HNO_3 and 3 mL of 37% HCl in a microwave oven (Multiwave GO, Anton Paar GmbH) at 180 °C for 60 min.

Digested samples were further diluted with ultrapure water and analyzed by ICP-MS (Agilent 7700 ×) except for Fe which was analyzed by MP-AES (Agilent 4210). During the ICP-MS analysis, internal standards were added: ^{115}In for Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se and ^{209}Bi for Pb. Blanks (*i.e.* ultrapure water adjusted to 2% HNO_3) were analyzed every 10 samples. Moreover, quality controls at 5 and 10 µg/L were added to check the performance of the analysis. The recovery was equal or above 86% for each element among all analyses performed by ICP-MS or MP-AES.

2.4.4. Method's limits of detection

The method's limits of detection were determined for each procedure (*i.e.* digestion or DGT handling) to account for sample contamination. For the acid digestion procedure, ultrapure water blanks were treated alongside samples with the procedure described in 2.4.3. Blank DGT devices were prepared in duplicate and treated alongside exposed devices during the "short" and "long term" experiments (see section 2.3.1). The method's limit of detection (MLD) and quantification (MLQ) were calculated according to IUPAC as the average plus three or ten times the standard deviation of the blanks for MLD and MLQ, respectively.

3. Results and discussions

3.1. Sample characterization

The characteristics (*i.e.* pH, TS, VS, TSS and VSS) of the samples collected for the short and long term experiments are summarized in Table S6. For each parameter, the difference in percentage is low (ranging from 4% to 7%).

The total and dissolved element concentrations of the samples is reported in Table S7. Dissolved element concentrations were below the MLQ except for As, Fe and Mn. A small discrepancy between the samples is observed for the dissolved Fe (9% difference) whereas a high discrepancy for the dissolved As (75% difference) and Mn (31% difference). Regarding the total element concentrations, only Se is not quantified in the samples. A small discrepancy is observed for Fe and Mn (≤ 9% difference) between the samples, whereas a discrepancy higher than 10% is observed for the other elements.

3.2. Validation of DGT principle

3.2.1. Steady state establishment

During the "short term" experiment, Cd, Cu, Mo and Pb were below the MLD whereas Al, Cr (III) and Se were below the MLQ of DGT deployment. Therefore, these elements are not discussed further in this section. According to DGT theory, steady state is rapidly established in the sampler (≤ 1 h, [19]) and the accumulated mass should behave linearly over time. The mass of elements accumulated over time on the Chelex and Zr-DGTs is reported in Fig. 2. We

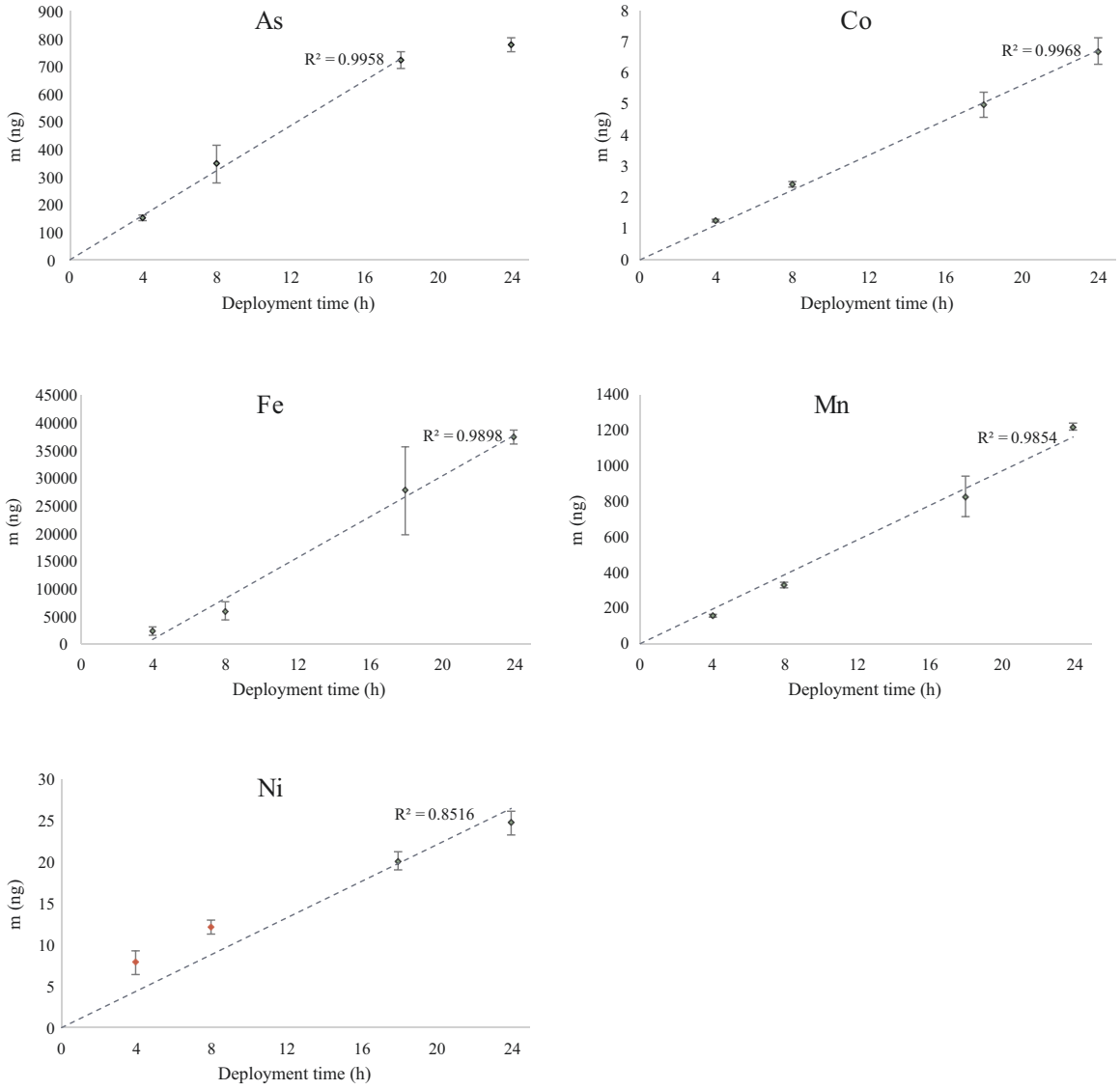


Fig. 2. The mass of elements accumulated on DGT samplers at different time of deployment during the "short term" experiment. In red, values between MLD_{DGT} and MLQ_{DGT}.

observe a linear accumulation trend from 0 to 24 h for Co, Mn and Ni. Therefore, the system (DGT-digestate) is rapidly in steady state and Eq. (2) holds for these elements regardless of the deployment time (until 24 h at least). We also observe a linear accumulation trend for As and Fe from 0 to 18 h and from 4 to 24 h, respectively. For As, it indicates that the steady state is rapidly reached and that Eq. (2) holds up to 18 h deployment. Deviation from linearity after 18 h is likely caused by competing effect. Indeed, Zr-binding gels are known to bind both As and P [20] that are chemical analogous (in the form of arsenate AsO_4^{3-} and phosphate PO_4^{3-}). Consequently, P could have replaced As on the binding gel. This hypothesis is supported by data shown in Fig. S1 where P displays the same linear behavior as As, but its accumulated mass on the Zr-binding gel was about 40-fold higher than As up to 24 h deployment time.

Fe presents a unique behavior since we observed linearity only after 4 h, indicating delayed establishment of steady state in the sampler. Such behavior can be explained by the properties of Fe complexes (partially labile complexes) or by interactions between Fe and the diffusive gel [21]. Such properties indicate that Eq. (2) does not hold at 4 h deployment and its use will result in an underestimation of C_{DGT}. Indeed, we calculated C_{DGT} from the regression line and compared to the value estimated with Eq. (2) using 4 and 24 h deployment and we found that C_{DGT} is highly underestimated at 4 h (*i.e.* 70% less) than 24 h deployment (*i.e.* 16% less).

We observed the establishment of steady state in the samplers for all the quantified elements, therefore the principle of DGT are validated for short deployments (≤ 24 h) in the studied digestate matrix. However, the non-significant accumulation of Al, Cd, Cu, Cr (III), Mo, Pb and Se during this "short term" experiment suggests that these elements may be countered by deploying the DGT samplers longer.

3.2.2. *Optimization of the deployment time*

To overcome the above mentioned limits of DGT samplers' deployment time, a "long term" experiment was performed. Increasing the deployment time up to 216 h did not enable the detection of labile Cd, Cu and Mo. Indeed, the concentration of these elements under labile form are lower than 0.4, 70 and 20 ng/L, respectively (MLD for 216 h DGT deployment).

The results of the "short" and "long term" experiments for the other studied elements are shown in Fig. 3. Except for As, Mn, Pb and Se, all quantified elements show linear accumulation over time up to 48 h (Cr (III), Fe, Ni), 72 h (Al) or 144 h (Co). Labile concentration of these elements can be therefore calculated with Eq. (2) using deployment time up to the above-mentioned values. Linearity breaks can result from

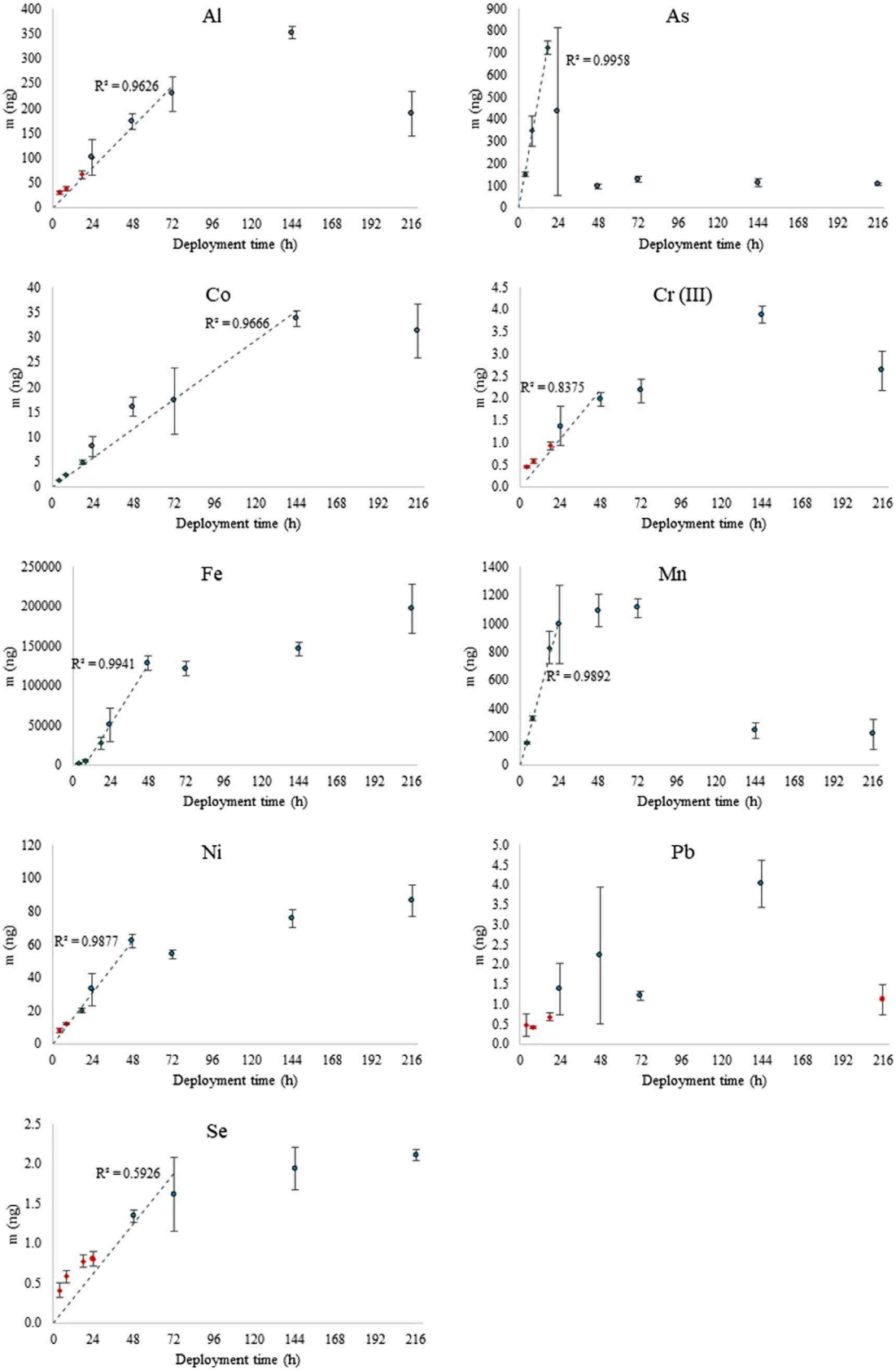


Fig. 3. Accumulated mass of elements *versus* deployment time during the "short" (green rhombus) and "long term" (blue circles) experiments. In red, values between MLD_{DGT} and MLQ_{DGT}. The 24 h point is an average between the two experiments.

the formation of pockets of gas observed between the DGT membrane and the diffusive gel (Fig. S2) that reduce the effective surface area of the DGT samplers. These pockets of gas likely derive from endogenous microorganisms. However, such hypothesis only holds for deployment times longer than 144 h since it should not be element dependent. For shorter deployment times, saturation of the binding gel appears a more realistic hypothesis. When saturation is reached, the accumulated mass of these elements rapidly decreases because of competing effect between elements. For example, the competing effect of Mg (element likely present in high amount in sewage sludge [2]) to Mn binding on the Chelex resin gel was studied by Jiménez-Piedrahita et al. [22]. Our results show that Mn does not accumulate linearly during the "long term" experiment (after 24 h).

Finally, increasing the deployment time enabled the quantification of Al, Cr (III), Pb and Se in samplers compared to the "short term" experiment. However, Pb does not linearly accumulate over time and quantification of labile concentration using Eq. (2) could be inappropriate. Moreover, the quantified Pb values are close to MLQ of DGT (from 1 to 3 fold). Such associated uncertainty can explain the nonlinear accumulation of Pb.

During the "short term" experiment, we observed an accumulation of As in the samplers over time, whereas not anymore during the "long term" experiment. Such behavior is consistent with the competing effect of P already highlighted and discussed in section 3.2.1.

Regarding Se, we cannot state that its accumulation trend is linear after 24 h deployment time ($R^2 < 0.6$). Consequently, this element cannot be correctly estimated using Eq. (2).

3.2.3. Impact of digestate matrix on accumulated labile elements

To check the interference of the digestate matrix on the trace elements accumulation by DGT samplers, some diffusive gels were pre-exposed for 24 h to the digestate before deployment in a well-defined spiked solution as described in 2.3.2. Since As (III) and Mo (VI) were below the MLQ of the DGT blanks, these elements are not further discussed in this section.

The mass of the elements accumulated by the control and "soiled" DGT samplers are presented in Fig. 4.

Except for Se (IV) and Cd (II), we observed that the accumulated mass of the elements measured by the control DGT samplers is significantly higher ($p < 0.05$) than the one measured by the "soiled" DGT samplers. In particular, the "soiled" DGT devices accumulates 11%, 18%, 24%, 28% less Co (II), Ni (II), Pb (II), Cu (II), respectively, compared to the control DGT devices. Such low accumulation could be even more pronounced in the digested sludge since its pH is higher than the one measured in the spiked solution of this study ($4 < pH < 6$, Table S1). A high pH is favorable for element binding to organic matter [23], at least for cations. In fact, organic matter is known to diffuse within diffusive gels [16,24–26]. We hypothesize that organic matter accumulated on the diffusive gel during pre-exposure and promoted element sorption onto the gel, resulting in a delay of element diffusion

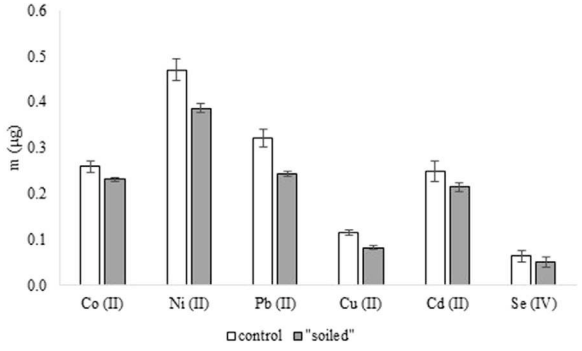


Fig. 4. Accumulated mass of trace elements by the control and "soiled" DGT samplers in 4 h deployment time.

Table 1
DGT method limit of detection (MLD_{DGT}) and quantification (MLQ_{DGT}) for 24 h deployment at 19 °C (average of recorded values during all deployments). The values are calculated using Eq. (2). The ratio between the MLQ for dissolved elements and the MLQ_{DGT} is also reported.

Element	MLD_{DGT} (µg/L)[a]	MLQ_{DGT} (µg/L)[b]	Ratio $MLQ_{dissolved}$ / MLQ_{DGT}
Al	2	4	1197
As	0.2	0.4	247
Cd	0.004	0.009	1288
Co	0.004	0.008	1383
Cr (III)	0.04	0.08	1707
Cu	0.7	2	543
Fe	0.9	2	898
Mn	0.1	0.3	320
Mo	0.2	0.4	74
Ni	0.3	0.7	852
Pb	0.02	0.04	4719
Se	0.02	0.04	35,742

[a] MLD = average blanks + 3σ blanks (n = 10).
[b] MLQ = average blanks + 10σ blanks (n = 10).

as already observed by Davison et al. [27] for Cu with river or soil organic matter.

Here, we showed that DGTs pre-exposure to the matrix of the digestate lowers the accumulation of most of the studied trace elements, leading to underestimation of the labile element concentrations in the medium.

3.3. DGT as a fractionation tool in digestates

3.3.1. Sensitivity of DGT method

The limit of detection and quantification of the method for DGT (MLD_{DGT} and MLQ_{DGT}) are given in Table 1. Compared to the instrumental limit of quantification (which only counts for the analytical sensitivity of the ICP-MS or MP-AES), the MLQ_{DGT} is at least two times higher (data not shown), meaning that some contamination of the samplers occurred during the samplers handling.

Additionally, we compared the MLQ_{DGT} to MLQ for dissolved element ($MLQ_{dissolved}$, Table 1). It arises that DGT greatly increased the sensitivity for element monitoring in the digested sludge than the conventional method (*i.e.* dissolved elements measurement). In particular, the MLQ_{DGT} for Al, Cd, Co, Cr (III), Pb and Se is more than 1000 lower than the $MLQ_{dissolved}$. For the other elements the ratio decreases in the following order Fe > Ni > Cu > Mn > As > > Mo. This high sensitivity is inherent to the sampling method since DGTs concentrate analytes whereas dissolved elements measurement requires acid digestion of the sample and subsequently its dilution. However, we must stress that both methods do not target the same chemical fraction since the labile fraction targeted by DGT represents only a part of the dissolved elements.

Besides, from a monitoring point of view, DGT appears a very interesting method since it allowed to quantify several of the labile elements during the experiments (Table S8) whereas it was not possible for most dissolved elements (Table S7). Therefore, we consider DGT as a sensitive method to monitor trace elements in digested sludge.

3.3.2. Fractionation with restricted gels in digestate matrix

A comparison between the labile concentrations of trace elements measured in DGT samplers with restricted and standard gels is reported

Table 2
The ratio between C_{DGT} measured in DGT samplers with restricted gel and the DGT samplers with standard gel.

	Al	As	Co	Cr(III)	Fe	Mn	Ni
$C_{labile\ restricted}/C_{labile\ standard}$	0.9	1.3	1.1	1.1	0.7	1.1	1. 3

in Table 2. Cu, Mo, Pb, Cd and Se are not shown because their concentration was below the MLQ_{DGT}.

Statistical analysis indicates that the labile concentration of Al, Co, Cr (III) and Mn measured by the DGT samplers with restricted gels is not significantly different from the one measured with standard gels ($p > 0.05$). It means that no large labile complexes of these elements are present in the studied digestate.

However, the labile concentration of Fe was significantly lower ($p < 0.01$) when measured with restricted gels (70% less) than standard gels, indicating the presence of some large labile Fe complexes (*i.e.* size > 1 nm).

Surprisingly, a significant higher concentration of labile As and Ni was estimated with restricted gels ($p < 0.02$) than standard gels. Such results are not consistent since restricted gels have smaller pore size (*i.e.* < 1 nm) than standard gels (*i.e.* > 5 nm) and it should not allow diffusion of a higher amount of labile elements. Such discrepancy could derive by the use of a non-adapted D value for the restricted gels. In fact, the values reported in Table S5 for D in the restricted gel are estimated in synthetic inorganic solutions, whereas in this study we demonstrated that the diffusion of trace elements is affected by the matrix of digestate. Therefore, we do not exclude that D in the restricted gel could be different in our sample compared to the D estimated in synthetic inorganic solutions. Finally, the interest of size fractionation with restricted gels foreseen above still have to be demonstrated.

3.4. Practical implementation for other digestate samples

In the studied digestate, the “short” and “long term” experiments revealed the following optimal deployment times for each element (Fig. 5):

A 24 h deployment appears a good compromise to allow quantification of most elements. However, these results cannot be generalized to any digestate sample given the variable composition of digestate in terms of trace elements and organic compounds which may interfere with elements’ accumulation in DGTs. Therefore, preliminary tests to optimize the deployment time are strongly recommended. In general, we advise to avoid long deployment time because saturation of the binding gel can occur due to the presence of other major compounds. Very short deployment time (*i.e.* < 4 h) should also be avoided, since the mass of trace elements may not accumulate in the device or the steady state is not established.

The studied digestate matrix altered accumulation of labile elements in DGT devices by 10–30% for Co (II), Ni (II), Pb (II), Cu (II). Such alteration was due to diffusion of organic matter in the sampler from the digestate matrix. This behavior is probably expected in most digestate samples given their high organic matter content [28,29]. Further studies are needed to determine the diffusion rate of trace elements in the presence of digestate matrix. From such work one should be able to correct for matrix effect with the aim to accurately determine labile trace elements concentrations. Unless this, it is safe to limit interpretation of labile concentration established with DGTs to general trends (*e.g.* evolution over time, order of magnitude) in order to limit misinterpretation of the absolute DGT labile trace elements concentrations.

Finally, size fractionation by coupling the restricted and standard gels was investigated in this study. Our results show the presence of large labile complexes for Fe (> 1 nm) and small labile complexes for Al, Co, Cr (III) and Mn (< 1 nm). However, these results must be confirmed and cannot be generalized at this stage.

3.5. Interpretation of DGT fractionation

One of the main objective when performing trace element fractionation is to predict their bio-accessibility. The DGT technique demonstrated to perform well mostly in natural waters and soils [7]. Currently, data regarding the relationship between DGT-labile element concentrations and their bio-accessibility in digestate are very sparse. To our knowledge, only the study of Bourven et al. [8] addressed this topic. They showed, in the context of whey anaerobic digestion, that DGT-labile Cd content is linked to the initial alteration of biogas production and enzymatic activities (*i.e.* β-galactosidase and TTC-dehydrogenase). However, such correlation was absent after 21 days of anaerobic digestion. DGT based fractionation of Cd appears, therefore, encouraging to predict its bio-accessibility, but not straightforward. Similar works could be performed for several trace elements and in various digestates. Therefore, new studies are required to fully establish the extent to which DGT fractionation can be used to predict elements bio-accessibility in digestates.

4. Conclusions

This study investigated the potential of DGT as a fractionation tool for trace elements in digested sewage sludge. Ensuring reliability of sampling is a prerequisite to the further use of DGT in digestate matrices. Our results suggest that DGT-labile trace elements sampling in digestate is feasible providing the deployment time is carefully tested and interpretation is limited to general trends (*e.g.* evolution over time, order of magnitude).

This study also showed that the DGT technique increases the sensitivity of trace elements monitoring compared to the dissolved element measurement by acid digestion. Moreover, DGT technique does not require sample treatment such as liquid-solid separation by centrifugation, preventing changes in trace elements speciation. These advantages over other fractionation methods already open a wide field of investigation for trace elements speciation in digestates.

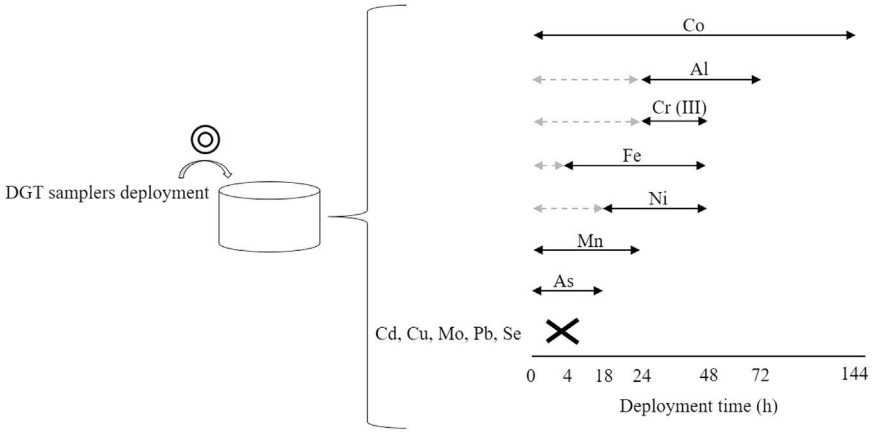


Fig. 5. Suitable deployment times for the studied digested sludge.

Acknowledgements

Patrice Fondanèche is acknowledged for his assistance in the laboratory, especially during ICP-MS and MP-AES analyses.

This project was sponsored from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 643071.

Conflict of interest

The authors declare no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2018.09.033.

References

[1] E.D. van Hullebusch, G. Guibaud, S. Simon, M. Lenz, S.S. Yekta, F.G. Fermoso, R. Jain, L. Duester, J. Roussel, E. Guillon, U. Skyllberg, C.M.R. Almeida, Y. Pechaud, M. Garuti, L. Frunzo, G. Esposito, C. Carliell-Marquet, M. Ortner, G. Collins, Methodological approaches for fractionation and speciation to estimate trace element bioavailability in engineered anaerobic digestion ecosystems: an overview, Crit. Rev. Environ. Sci. Technol. 46 (2016) 1324–1366, https://doi.org/10.1080/10643389.2016.1235943.

[2] R. Zufiaurre, A. Olivar, P. Chamorro, C. Nerin, A. Callizo, Speciation of metals in sewage sludge for agricultural uses, Analyst 123 (1998) 255–259, https://doi.org/10.1039/a705168i.

[3] A.F.M. Braga, M. Zaiat, G.H.R. Silva, F.G. Fermoso, Metal fractionation in sludge from sewage UASB treatment, J. Environ. Manag. 193 (2017) 98–107, https://doi.org/10.1016/j.jenvman.2017.01.070.

[4] N. Zhu, Q. Li, X. Guo, H. Zhang, Y. Deng, Sequential extraction of anaerobic digestate sludge for the determination of partitioning of heavy metals, Ecotoxicol. Environ. Saf. 102 (2014) 18–24, https://doi.org/10.1016/j.ecoenv.2013.12.033.

[5] J.R. Bacon, C.M. Davidson, Is there a future for sequential chemical extraction? Analyst 133 (2008) 25–46, https://doi.org/10.1039/b711896a.

[6] P.M. Thanh, B. Ketheesan, Z. Yan, D. Stuckey, Trace metal speciation and bioavailability in anaerobic digestion: a review, Biotechnol. Adv. 34 (2016) 122–136, https://doi.org/10.1016/j.biotechadv.2015.12.006.

[7] H. Zhang, W. Davison, Use of diffusive gradients in thin-films for studies of chemical speciation and bioavailability, Environ. Chem. 12 (2015) 85–101, https://doi.org/10.1071/EN14105.

[8] I. Bourven, M. Casellas, R. Buzier, J. Lesieur, J.F. Lenain, A. Faix, P. Bressolier, C. Maftah, G. Guibaud, Potential of DGT in a new fractionation approach for studying trace metal element impact on anaerobic digestion: the example of cadmium, Int. Biodeterior. Biodegrad. 119 (2017) 188–195, https://doi.org/10.1016/j.ibiod.2016.11.007.

[9] M. Takashima, E. Nakakihara, R. Ikemoto, Effects of thermal pretreatment and trace metals on high- rate thermophilic anaerobic digestion of sewage sludge, J. Environ. Eng. 144 (2018) 3–6, https://doi.org/10.1061/(ASCE)EE.1943-7870.0001340.

[10] H. Ernstberger, H. Zhang, W. Davison, Determination of chromium speciation in natural systems using DGT, Anal. Bioanal. Chem. 373 (2002) 873–879, https://doi.org/10.1007/s00216-002-1370-3.

[11] H. Zhang, W. Davison, B. Knight, S. Mcgrath, In situ measurements of solution concentrations and fluxes of trace metals in sells using DGT, Environ. Sci. Technol. 32 (1998) 704–710, https://doi.org/10.1021/es9704388.

[12] D. Devillers, R. Buzier, S. Simon, A. Charriau, G. Guibaud, Simultaneous measurement of Cr(III) and Cr(VI) in freshwaters with a single Diffusive Gradients in Thin Films device, Talanta 154 (2016) 533–538, https://doi.org/10.1016/j.talanta.2016.04.009.

[13] H. Zhang, W. Davison, Diffusional characteristics of hydrogels used in DGT and DET techniques, Anal. Chim. Acta 398 (1999) 329–340, https://doi.org/10.1016/S0003-2670(99)00458-4.

[14] S. Scally, W. Davison, H. Zhang, Diffusion coefficients of metals and metal complexes in hydrogels used in diffusive gradients in thin films, Anal. Chim. Acta 558 (2006) 222–229, https://doi.org/10.1016/j.aca.2005.11.020.

[15] D. Devillers, R. Buzier, A. Charriau, G. Guibaud, Improving elution strategies for Chelex®-DGT passive samplers, Anal. Bioanal. Chem. 409 (2017) 7183–7189, https://doi.org/10.1007/s00216-017-0680-4.

[16] H. Zhang, W. Davison, Performance characteristics of diffusion gradients in thin films for the in situ measurement of trace metals in aqueous solution, Anal. Chem. 67 (1995) 3391–3400, https://doi.org/10.1021/ac00115a005.

[17] E.W. Lemmon, M.O. McLinden, D.G. Friend, Thermophysical Properties of Fluid Systems, in: P.J. Linstrom, W.G. Mallard (Eds.), Natl. Institue Stand. Technol., Gaithersburg MD, 2010. ⟨http://webbook.nist.gov/chemistry/fluid/⟩.

[18] A.H. Shiva, P.R. Teasdale, W.W. Bennett, D.T. Welsh, A systematic determination of diffusion coefficients of trace elements in open and restricted diffusive layers used by the diffusive gradients in a thin film technique, Anal. Chim. Acta 888 (2015) 146–154, https://doi.org/10.1016/j.aca.2015.07.027.

[19] M.H. Tusseau-Vuillemin, R. Gilbin, M. Taillefert, A dynamic numerical model to characterize labile metal complexes collected with diffusion gradient in thin films devices, Environ. Sci. Technol. 37 (2003) 1645–1652, https://doi.org/10.1021/es025839o.

[20] S. Ding, D. Xu, Q. Sun, H. Yin, C. Zhang, Measurement of dissolved reactive phosphorus using the diffusive gradients in thin films technique with a high-capacity binding phase, Environ. Sci. Technol. 44 (2010) 8169–8174, https://doi.org/10.1021/es1020873.

[21] W. Davison, H. Zhang, Progress in understanding the use of diffusive gradients in thin films (DGT) back to basics, Environ. Chem. 9 (2012) 1–13, https://doi.org/10.1071/EN11084.

[22] M. Jiménez-Piedrahita, A. Altier, J. Cecilia, J. Puy, J. Galceran, C. Rey-Castro, H. Zhang, W. Davison, Extending the use of diffusive gradients in thin films (DGT) to solutions where competition, saturation, and kinetic effects are not negligible, Anal. Chem. 89 (2017) 6567–6574, https://doi.org/10.1021/acs.analchem.7b00704.

[23] P. Fletcher, P.H.T. Beckett, The chemistry of heavy metals in digested sewage sludge-II. Heavy metal complexation with soluble organic matter, Water Res. 21 (1987) 1163–1172, https://doi.org/10.1016/0043-1354(87)90167-9.

[24] P.L.R. Van Der Veeken, H.P. van Leeuwen, DGT/DET gel partition features of humic acid/metal species, Environ. Sci. Technol. 44 (2010) 5523–5527, https://doi.org/10.1021/es100861t.

[25] P.L.R. Van Der Veeken, P. Chakraborty, H.P. van Leeuwen, Accumulation of humic acid in DET/DGT gels, Environ. Sci. Technol. 44 (2010) 4253–4257, https://doi.org/10.1021/es100510u.

[26] B. Docekal, V. Rezacova-Smetkova, H. Docekalova, Effect of humic acid on metal uptake measured by diffusive gradients in thin films technique, Chem. Pap. Zvesti 59 (2005) 298–303.

[27] W. Davison, C. Lin, Y. Gao, H. Zhang, Effect of gel interactions with dissolved organic matter on DGT measurements of trace metals, Aquat. Geochem. 21 (2015) 281–293, https://doi.org/10.1007/s10498-014-9244-9.

[28] E. Tampio, T. Salo, J. Rintala, Agronomic characteristics of five different urban waste digestates, J. Environ. Manag. 169 (2016) 293–302, https://doi.org/10.1016/j.jenvman.2016.01.001.

[29] C. Teglia, A. Tremier, J.L. Martel, Characterization of solid digestates: part 2, assessment of the quality and suitability for composting of six digested products, Waste Biomass Valoriz. 2 (2011) 113–126, https://doi.org/10.1007/s12649-010-9059-x.

Supporting information for
Assessment of the DGT technique in digestate to fraction twelve trace elements

Andreina Laera[a,b,c], Rémy Buzier[b]*, Gilles Guibaud[b], Giovanni Esposito[c], Eric D. van Hullebusch[a,d]

[a] University of Paris-Est, Laboratoire Géomatériaux et Environnement (EA 4508), UPEM, 77454 Marne-la-Vallée, France, andreina.laera@u-pem.fr

[b] University of Limoges, PEIRENE, Equipe Développement d'indicateurs ou prévision de la qualité des eaux, URA IRSTEA, 123 Avenue Albert Thomas, 87060 Limoges Cedex, France

[c] University of Cassino and Southern Lazio, Department of Civil and Mechanical Engineering, via Gaetano di Biasio 43, 03043 Cassino (FR), Italy

[d] IHE Delft Institute for Water Education, Department of Environmental Engineering and Water Technology, PO Box 3015, 2601 DA Delft, The Netherlands

*Corresponding author: remy.buzier@unilim.fr

Contents

Experimental

Restricted gel preparation

The restricted diffusive gel was made with 3.55 mL of 40% (m/V) acrylamide mixed with 1 mL of 7.5% (m/V) bis-acrylamide and completed up to 10 mL with ultrapure water. Later, 200 µL of 10% (m/V) freshly prepared ammonium persulfate (Fisher Scientific) and 8 µL of TEMED (Aldrich) were mixed with 10 mL of the gel solution. To cast the gel, two glass plates were separated by a 0.75 mm thick Teflon spacer, and the gel solution was poured between the plates. The plates were placed in an oven at 45°C for 45 min to allow rapid polymerization. Compared to standard diffusive gel, restricted gel is brittle, therefore the gel plates were cut into round disks and rinsed with ultrapure water at least five times during 24 hours to remove any impurities from the gels. The gels were then stored in 0.01 M $NaNO_3$ at 4°C. According to Zhang and Davison [1], standard diffusive gel has a pore size of >5 nm, whereas a restricted gel <1 nm.

Table S1. Parameters measured at the beginning and at the end of the experiment to assess the interference of digestate matrix on trace elements accumulation.

		Measured parameters	
Solution of 10^{-2} M NaCl +	cations	pH_{start}	4.2
		pH_{end}	4.4
		T_{start}	19 °C
		T_{end}	22 °C
Solution of 10^{-2} M NaCl +	anions	pH_{start}	5.5
		pH_{end}	6.1
		T_{start}	19 °C
		T_{end}	22 °C

Table S2. Concentration of the elements added in the two solutions to study the interference of the digestate matrix on the trace elements accumulation. Concentrations were chosen to be either quantifiable or in the same order of magnitude compared to the studied digestate samples.

	Added element	µg/L
Solution of 10^{-2} M NaCl + cations	Co (II)	71
	Ni (II)	128
	Pb (II)	72
	Cu (II)	30
	Cd (II)	67
Solution of 10^{-2} M NaCl + anions	As (III)	22
	Se (IV)	7
	Mo (VI)	7

Table S3. Elution factors f_e from the literature with elution conditions similar to this work.

	Elution factor	Reference
Al	0.85	[2]
As	0.70	Result not published
Cd	0.85	[2]
Co	0.85	[2]
Cr	0.80	[2]
Cu	0.85	[2]
Fe	0.70	[3]
Mn	0.82	[3]
Mo	0.86	Result not published
Ni	0.85	[2]
P	0.95	[4]
Pb	0.85	[2]
Se	0.86	Result not published

Table S4. Coefficients of diffusion in a standard diffusive gel taken from the literature and used in this study. All values are referred to 25°C.

	$D_{standard}$ (cm^2/sec)
Al	$4.75 \cdot 10^{-6}$[a]
As	$8.29 \cdot 10^{-6}$[b]
Cd	$6.09 \cdot 10^{-6}$[a]
Co	$5.94 \cdot 10^{-6}$[a]
Cr (III)	$5.05 \cdot 10^{-6}$[a]
Cu	$6.23 \cdot 10^{-6}$[a]
Fe	$6.11 \cdot 10^{-6}$[a]
Mn	$5.85 \cdot 10^{-6}$[a]
Mo	$6.62 \cdot 10^{-6}$[c]
Ni	$5.77 \cdot 10^{-6}$[a]
P	$6.05 \cdot 10^{-6}$[a]
Pb	$8.03 \cdot 10^{-6}$[a]
Se	$7.07 \cdot 10^{-6}$[b]

[a] Reference: http://www.dgtresearch.com/diffusion-coefficients/

[b] Reference: [5]

[c] Reference: [6]

Table S5. The ratio of the restricted diffusion coefficients to the standard diffusion coefficients estimated by experimental works in different laboratories.

Element	$D_{restricted}/D_{standard}$
Al	0.72[b]; 0.68[c]
As	0.71[b]; 0.71[c]
Cd	0.62[a]; 0.73[b]; 0.72[c]
Co	0.76 [b]; 0.71[c]
Cr	-
Cu	0.70[a]; 0.78[b]; 0.72[c]
Fe	-
Mn	0.78[b]; 0.71[c]
Mo	0.68[b]; 0.71[c]
Ni	0.69[a]; 0.72[b]; 0.72[c]
Pb	0.72[a]; 0.72[b]; 0.73[c]
Se	-

[a] Estimated by the diffusion cell method. Reference [7]

[b] Estimated by the DGT time-series method at pH 4. Reference [8]

[c] Estimated by the diffusion cell method at pH 4. Reference [8]

Table S6. Characteristics of the digested sewage sludge (DSS) collected at different time for the "short" and "long term" experiments. Except for the pH, results are mean of duplicates ± standard deviation. These measurements were performed immediately after the samples collection.

	pH	TSS (g/L)	VSS (wt%)	TS (wt%)	VS (wt%)
DSS collected for the "short term" experiment	7.7	33.5±0.3	66.0±0.1	3.4±0.1	64.9±0.2
DSS collected for the "long term" experiment	7.3	31.7±0.2	69.1±0.0	3.3±0.0	69.3±0.2

Table S7. Dissolved and total elements concentration in the digested sewage sludge (DSS) for the "short" and "long term" experiments.

	DSS collected for the "long term" experiment		DSS collected for the "short term" experiment	
	Dissolved† [µg/L $_{sludge}$]	Total‡ [µg/g $_{TS\ initial}$]	Dissolved† [µg/L $_{sludge}$]	Total‡ [µg/g $_{TS\ initial}$]
Al	<4327*	13311±778	<4327*	9619±2380
As	136±23	100±6	300±14	126±21
Cd	<11*	1.94±0.13	<11*	1.21±0.17
Co	11±2	7±1	<11*	5±1
Cr	<139*	72±4	<139*	30±5
Cu	<906*	449±24	<906*	296±52
Fe	16789±2559	57006±5449	18414±599	62396±12245
Mn	247±1	601±42	336±18	656±116
Mo	<32*	7.1±0.3	<32*	5.8±0.9
Ni	<585*	37±8	<585*	20±3
Pb	<179*	89±9	<179*	42±7
Se	<1298*	<18#	<1298*	<18#

†The dissolved elements concentration is the mean of duplicates taken at the beginning of the experiment ± standard deviation.

‡The total elements concentration is the mean of duplicates taken at the beginning and at the end of the experiment ± standard deviation.

*MLQ=average blanks ± 10*standard deviation blanks (n=10) expressed on the same concentration basis (µg/L $_{sludge}$) as those for the samples using 25 L/L $_{sludge}$ as conversion factor.

#MLQ=average blanks ± 10*standard deviation blanks (n=10) expressed on the same concentration basis (µg/g $_{TS\ initial}$) as those for the samples using 0.35 L/g $_{TS\ initial}$ as conversion factor.

Table S8. C_{DGT} of all studied elements for 24h deployment at 15°C (average of recorded values during the "short term" experiment). The values are calculated using Eq. (2). *MLQ for DGT deployment (average blanks+10σ blanks, n=10).

	C_{DGT} (µg/L)
Al	3.3±0.2
As	20.7±0.7
Cd	<0.009*
Co	0.25±0.02
Cr	<0.08*
Cu	<2*
Fe	1260±50
Mn	46.1±0.6
Mo	<0.4*
Ni	0.94±0.06
Se	<0.04*
Pb	<0.04*

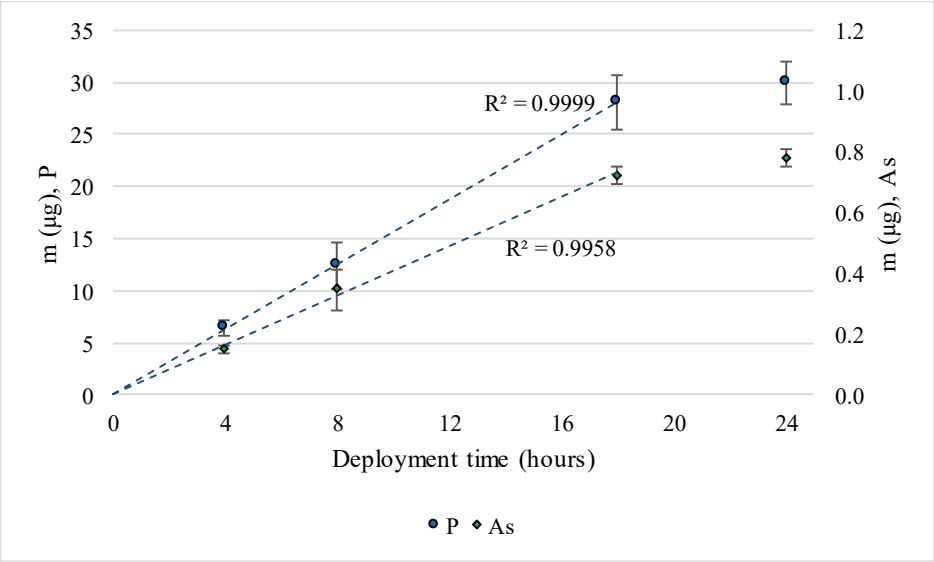


Figure S1. Comparison of accumulated mass of P and As on Zr-binding gels during the "short term" experiment. Analysis of P was performed using ICP-MS.

Chelex-DGT samplers (24h deployment time)

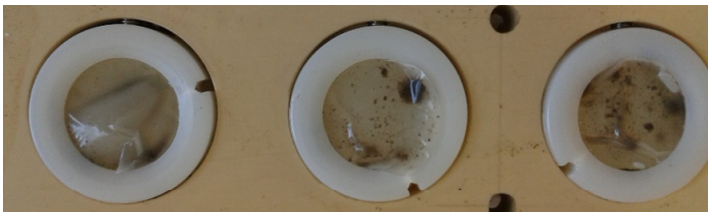
Chelex-DGT samplers (48h deployment time)

Zr-DGT samplers (48h deployment time)

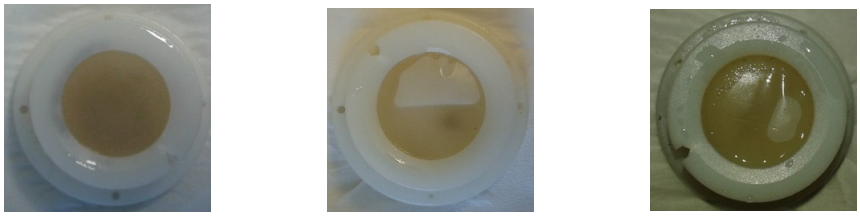
Chelex-DGT samplers (72h deployment time)

Zr-DGT samplers (72h deployment time)

Chelex-DGT samplers (144h deployment time)



Zr-DGT samplers (144h deployment time)



Chelex-DGT samplers (216h deployment time)

Zr-DGT samplers (216h deployment time)

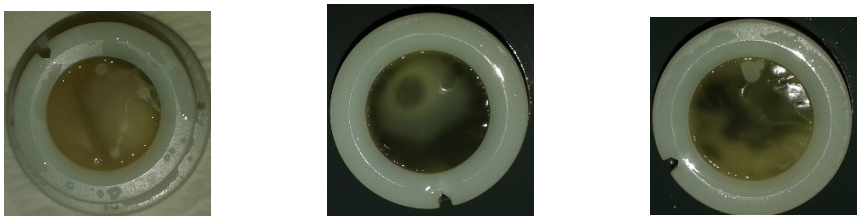


Figure S2. Images of Chelex and Zr-DGT samplers recovered at different deployment time during the "long term" experiment. Bigger pockets of gas from endogenous microorganisms are formed between the DGT membrane and the diffusive gel at increasing the deployment time.

References

[1] H. Zhang, W. Davison, Diffusional characteristics of hydrogels used in DGT and DET techniques, Anal. Chim. Acta. 398 (1999) 329–340. doi:10.1016/S0003-2670(99)00458-4.

[2] D. Devillers, R. Buzier, A. Charriau, G. Guibaud, Improving elution strategies for Chelex®-DGT passive samplers, Anal. Bioanal. Chem. 409 (2017) 7183–7189. doi:10.1007/s00216-017-0680-4.

[3] H. Zhang, W. Davison, Performance Characteristics of Diffusion Gradients in Thin Films for the in Situ Measurement of Trace Metals in Aqueous Solution, Anal. Chem. 67 (1995) 3391–3400. doi:10.1021/ac00115a005.

[4] S. Ding, D. Xu, Q. Sun, H. Yin, C. Zhang, Measurement of dissolved reactive phosphorus using the diffusive gradients in thin films technique with a high-capacity binding phase, Environ. Sci. Technol. 44 (2010) 8169–8174. doi:10.1021/es1020873.

[5] W.W. Bennett, P.R. Teasdale, J.G. Panther, D.T. Welsh, D.F. Jolley, New diffusive gradients in thin films technique for measuring inorganic arsenic and selenium (IV) using a titanium dioxide based adsorbent, Anal. Chem. 82 (2010) 7401–7407.

[6] Y. Wang, S. Ding, D. Xu, Y. Wang, Y. Li, M. Gong, C. Zhang, Simultaneous measurements of eight oxyanions using high-capacity diffusive gradients in thin films (Zr-Oxide DGT) with a high-efficiency elution procedure, Environ. Sci. Technol. 50 (2016) 7572–7580. doi:10.1021/acs.est.6b00206.

[7] S. Scally, W. Davison, H. Zhang, Diffusion coefficients of metals and metal complexes in hydrogels used in diffusive gradients in thin films, Anal. Chim. Acta. 558 (2006) 222–229. doi:10.1016/j.aca.2005.11.020.

[8] A.H. Shiva, P.R. Teasdale, W.W. Bennett, D.T. Welsh, A systematic determination of diffusion coefficients of trace elements in open and restricted diffusive layers used by the diffusive gradients in a thin film technique, Anal. Chim. Acta. 888 (2015) 146–154. doi:10.1016/j.aca.2015.07.027.

III

DISTRIBUTION TREND OF TRACE ELEMENTS IN DIGESTATE EXPOSED TO AIR: LABORATORY-SCALE INVESTIGATIONS USING DGT-BASED FRACTIONATION

by

Andreina Laera, Rémy Buzier, Gilles Guibaud, Giovanni Esposito & Eric D. van Hullebusch, 15 May 2019
Journal of Environmental Management 238, 159-165
Reproduced with kind permission by Elsevier.

Journal of Environmental Management 238 (2019) 159–165

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Distribution trend of trace elements in digestate exposed to air: Laboratory-scale investigations using DGT-based fractionation

Andreina Laera[a,b], Rémy Buzier[b,*], Gilles Guibaud[b], Giovanni Esposito[c], Eric D. van Hullebusch[d]

[a] University of Paris-Est, Laboratoire Géomatériaux et Environnement (EA 4508), UPEM, 77454, Marne-la-Vallée, France
[b] University of Limoges, PEIRENE, Equipe Développement d'indicateurs ou prévision de la qualité des eaux, URA IRSTEA, 123 Avenue Albert Thomas, 87060, Limoges Cedex, France
[c] University of Napoli "Federico II", Department of Civil, Architectural and Environmental Engineering, via Claudio 21, 80125, Napoli, Italy
[d] Institut de Physique du Globe de Paris, Sorbonne Paris Cité, Université Paris Diderot, UMR 7154, CNRS, F-75005, Paris, France

ARTICLE INFO

Keywords:
Metals
Metalloids
Digested sewage sludge
Fractionation
Diffusive gradients in thin films (DGT)
Speciation

ABSTRACT

The use of digestate as amendment for agricultural soils has already been proposed as an alternative to mineral fertilizers or undigested organic matter. However, little information is available concerning the effect of digestate atmospheric exposure on trace elements speciation and, consequently, on their mobility and bio-accessibility when digestate is stored in open tanks or handled before land spreading. In this study, we investigated at laboratory-scale the effect of digestate aeration on the distribution of Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se and W using the diffusive gradients in thin films technique (DGT)-based fractionation. For this purpose, experiments were performed to assess the variation in distribution between the labile, soluble and particulate fractions over time in digested sewage sludge during passive and forced aeration. Results showed that aeration promoted a dissolution of Al, As, Co, Cr, Cu, Fe, Mn, Mo and Pb, suggesting a possible increase in their mobility that may likely occur during storage in open tanks or handling before land spreading. Labile elements' fraction increased only during forced aeration (except for Fe and Mn), suggesting that their short-term bio-accessibility can increase only after significant aeration as the one assumed to occur when land spreading takes place.

1. Introduction

The use of digestate, a by-product of anaerobic digestion of organic residues (Möller and Müller, 2012), as amendment for agricultural soils and substitute of mineral fertilizers (Riva et al., 2016) is gaining importance as a result of the increasing use of biogas plants running on different organic feedstock (Scarlat et al., 2018). However, the presence of potentially hazardous trace elements (TEs) (*e.g.* cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn)) in digestate, may prevent its use in agriculture (Kupper et al., 2014; Tampio et al., 2016). The bio-accessibility of TEs not only depends on their total concentration but also on their speciation (Hooda, 2010). Therefore, screening of TEs speciation is required to assess the harm or benefit associated with digestate before land spreading (van Hullebusch et al., 2016).

According to the spreading season, digestate could be stored for several months (Plana and Noche, 2016) in open tanks (Boulamanti et al., 2013; Liebetrau et al., 2010). During storage in open tanks and handling before land spreading, digestate will be exposed to air. Such exposure will alter the anaerobic status of digestate which in turn may alter the speciation of TEs and consequently affect their mobility and bio-accessibility in the environment. Although no information is available, to the best of our knowledge, for digestate, Øygard et al. (2007) demonstrated that atmospheric exposure impacts on TEs' distribution in municipal solid waste landfill leachates. Therefore, new investigations are needed to assess the potential impact of digestate aeration on TEs speciation for risk assessment before land application.

Total element content in digestate is commonly determined after solubilization (usually acid digestion) with conventional methods for TEs analysis in liquids such as ICP-MS (Dragicevic et al., 2018a) and ICP-OES (Cao et al., 2018). The mobility and bio-accessibility of TEs in digestate are usually studied using different techniques such as sequential extractions like the modified Tessier method (Ortner et al., 2014) or extraction with deionized water only (Dragicevic et al., 2018b). Alternatively, the diffusive gradients in thin films technique (DGT) can be used to screen the presence of labile elements (*i.e.* the most readily bio-accessible form of TEs) (Zhang and Davison, 2015)

* Corresponding author.
E-mail addresses: andreina.laera@u-pem.fr, piedelibero89@gmail.com (A. Laera), remy.buzier@unilim.fr (R. Buzier).

https://doi.org/10.1016/j.jenvman.2019.02.120
Received 12 November 2018; Received in revised form 20 February 2019; Accepted 25 February 2019
0301-4797/ © 2019 Elsevier Ltd. All rights reserved.

into the environmental matrix. In particular, DGT-based fractionation was recently validated for digestate matrix (Laera et al., 2019). Compared to conventional fractionation techniques, DGT has the advantage of measuring the targeted elements *in situ* without affecting the sample and speciation of TEs (Vrana et al., 2005). Moreover, DGT technique increases the sensitivity of TEs monitoring compared to total acid-soluble measurements (Laera et al., 2019).

Here, the effects of aeration of digested sewage sludge on mobility and bio-accessibility of fourteen TEs were investigated to assess their fate before land spreading. The TEs investigated in this study are either under EU regulation for application of sewage sludge in agriculture (European Commission, 2016) (*i.e.* Cd, Cr, Cu, Ni and Pb), or selected based on previous studies (Dragicevic et al., 2018b; Hamnér and Kirchmann, 2015; Laera et al., 2019; Øygard et al., 2007) (*i.e.* Al, As, Co, Fe, Mn, Mo and Se). Antimony (Sb) and W were included because they could be present in sewage sludge (Fu and Tabatabai, 1988; Healy et al., 2016; McBride, 2003) and generate environmental issues due to their accumulation in plants (Arai, 2010; Charter et al., 1995).

In this study, the conventional particulate/soluble fractionation indicating potential TEs' mobility was implemented with a DGT-based fractionation procedure to monitor the most bio-accessible species. Experiments were performed at laboratory-scale to assess the time variation of labile, soluble and particulate TEs during passive and forced aeration of digestate. Results were discussed assuming that the experimental work can mimic digestate oxidation during storage in open tanks or handling before land spreading.

2. Material and methods

2.1. Digestate sample

Digested sewage sludge was collected from a municipal wastewater treatment plant located in Limoges (France). The digestate derived from activated sludge treated by a mesophilic anaerobic digestion process. About 18 L of sample was collected directly from a pipe before discharge in an open storage tank. The sample was collected in a polypropylene (PP) bucket up to maximum capacity and closed with a lid to limit sample exposure to open air. Once in the laboratory, the sample was stored at 4 °C for less than 24 h before starting the experiment.

2.2. DGT preparation

We used Chelex-DGTs for cationic species (Al, Cd, Co, Cr (III), Cu, Fe, Mn, Ni and Pb) and zirconia-DGTs (Zr-DGTs) for anionic species (As, Mo, Sb, Se and W). Each DGT consisted of a binding gel (Chelex or Zr), a diffusive gel and a filter membrane enclosed in a piston type holder (purchased from DGT Research, Lancaster, UK). Chelex binding gels were prepared according to the procedure described by Zhang et al. (1998), whereas Zr binding gels were made according to Devillers et al. (2016). Diffusive gels were standard polyacrylamide gels (15% acrylamide and 0.3% agarose-derived cross linker, 0.77 mm thick) prepared according to Zhang et al. (1998) and filter membranes were made of cellulose acetate (0.2 µm pore size, 0.12 mm thickness, Whatman, UK).

2.3. Experimental set-up

About 18 L of digested sludge were poured into a laboratory-scale PP tank placed under a fume hood and continuously stirred with an overhead plastic propeller at 30 rpm (Fig. S1) in order to control experimental conditions. Stirring allows optimizing air transfer within the digestate and therefore represents a "worst case scenario" compared to unstirred real scale tanks. A Tinytag data logger (TG-4100, Gemini Data Loggers, UK) was used to record the temperature in the sample every 10 min. The surface of the sample was exposed to air to promote oxidation of the sample during 10 weeks. The surface to volume ratio varied from 0.39 dm^{-1} (7.1 dm^2:18 L) to 0.51 dm^{-1} (7.1 dm^2:14 L) during the experiment because of multiple sample collection (see below). Therefore, passive aeration was progressively favored while the experiment continued. Then, aeration was enhanced during 2 supplementary weeks by introducing 4 micro-bubble air diffusers in the digested sludge. All diffusers were connected to air pumps (Newair or Optima) having airflow rates from 60 to 200 L/h.

Labile TEs were sampled by deploying three DGTs probes composed either of Chelex or Zr for 24 h in the digested sludge. We choose a 24 h deployment because it was shown previously to be a good compromise for the studied elements in digestate (Laera et al., 2019).

DGTs were deployed according to the following sequence (Fig. S1): every day for the 6 first consecutive days; once per week from week 2–10; twice per week for weeks 11 and 12. Blanks DGT devices were also prepared in duplicate and treated alongside exposed devices every week.

After DGTs' retrieval, we measured dissolved O_2, redox potential (E_h) and pH. Additionally, we collected an aliquot of sample to measure total and volatile solids (TS and VS), total and volatile suspended solids (TSS and VSS) and soluble TEs. Additionally, we monitored sulfate (SO_4^{2-}) concentration.

2.4. Analytical procedures

2.4.1. Physicochemical analysis

pH and E_h were measured with a Mettler Toledo pH meter and a Radiometer electrode, respectively. Dissolved oxygen was measured using a ProODO™ optical sensor (YSI). Each sampling time, about 90 mL of sample was collected in duplicate to measure the total solids (TS), volatile solids (VS), total suspended solids (TSS) and volatile suspended solids (VSS) according to the French standard AFNOR NF T90-105 method.

The supernatant recovered during the TSS and VSS analysis was conserved to determine soluble TEs (see Section 2.4.2.).

2.4.2. Sample treatment and trace elements analysis

Total elements' content was determined at the beginning and at the end of the experiment using 5 g of raw sample. Each sampling time, soluble elements' concentration was determined from the supernatant recovered during TSS determination. Supernatants and raw samples in duplicate were acid digested with 6 mL of 69% HNO_3 and 3 mL of 37% HCl in a microwave oven (Multiwave GO, Anton Paar GmbH) at 180 °C for 60 min.

TEs were analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700 ×) except for Fe which was analyzed by microwave plasma atomic emission spectroscopy (MP-AES, Agilent 4210). Blanks and quality controls at 5 and 10 µg/L were analyzed every 10 samples. The recovery was equal or above 86% for each element, except for Sb and W which was equal or above 79% and 76%, respectively, among all analyses.

2.5. Element's fractionations calculation

The fractionation procedure is presented in Fig. 1. Particulate elements' concentration was calculated by subtracting the soluble to the initial total elements content.

After retrieval from the digested sludge, DGT samplers were rinsed with ultrapure water and disassembled to recover the binding gels and determine labile elements concentration. The accumulated mass (m) was determined following elution of binding gels in 2 mL of 1 M HNO_3 or 5×10^{-3} M NaOH and 0.5 M H_2O_2 for 24 h for Chelex and Zr-binding gels, respectively (see Table S1 for elution yields). The concentration of labile TEs, $C_{DGT,}$ was then derived using Eq. (1) based on Fick's first law (Zhang and Davison, 1995):

$$C_{DGT} = \frac{m \times \Delta_{MDL}}{D \times t \times A} \quad (1)$$

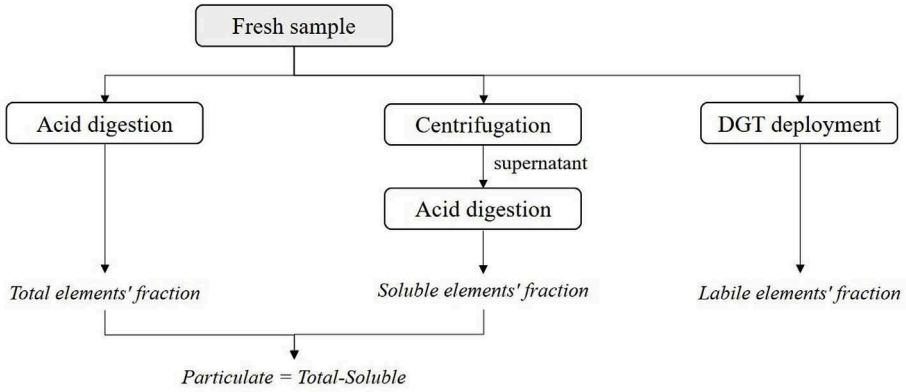


Fig. 1. Fractionation procedure adopted in this study to estimate total, soluble, particulate and labile elements' fractions.

where Δ_{MDL} is the thickness of the material diffusion layer (*i.e.* diffusive gel plus membrane, 0.89 mm), t is the time of DGT samplers' exposure in the sludge (24 h), D is the coefficient of diffusion of the considered element and A is the geometric area of the DGT holder window (3.14 cm^2). D values were taken from literature (Table S2) and corrected for the average temperature recorded during each deployment using Stokes–Einstein relation (Zhang and Davison, 1999).

The method's limits of detection and quantification (namely MLD and MLQ for total and soluble elements or MLD_{DGT} and MLQ_{DGT} for labile elements) are displayed in Tables S3 and S4.

3. Results and discussion

3.1. Sample characterization

The characteristic of the digested sewage sludge (TS, VS and water concentration) are presented in Fig. S2. The results show that the water concentration and the VS content is nearly constant throughout the experiment. In particular, the average water content was 96.2% ± 1.6 and the average VS content was 63.9% ± 1.3. Moreover, the average pH of the digested sludge was 7.8 ± 0.3 and the E_h was below −50 mV, whatever the aeration of the sludge. The latter is shown in Fig. S3.

The total elements concentration in the digested sludge is shown in Table S5. Except for Cd, Mo and Ni, the concentration of total elements is not significantly different ($p > 0.05$) at the beginning and at the end of the experiment. For total Cd, Mo and Ni content the difference was significant and could derive from an artifact caused by multiple sampling during the experiment if these elements were not homogenously distributed in the sludge.

3.2. Particulate and soluble concentrations of elements

Soluble concentrations of Cd, Ni, Sb, Se and W were below the method's limits of detection or quantification (*i.e.* lower than 12, 721, 102, 1077 and 69 µg/L, respectively) during the whole experiment and the impact of aeration on their distribution cannot be discussed. For the other elements (Fig. S4), three different trends were observed. An example of each trend is given in Fig. 2. Fe and Mn showed limited variations of their particulate and soluble concentrations during the first 15 days of passive aeration. Then, their soluble concentrations doubled up to the 66th day of aeration with a limited influence on their particulate concentration. From the 76th day of passive aeration and during the two weeks of forced aeration, the soluble concentration of Fe and Mn rapidly doubled. This rapid release in solution generated a slight decrease in particulate Fe (*i.e.* 4% less) and Mn (*i.e.* 5% less). Soluble concentrations of Al, Co, Cr, Cu, Mo and Pb were below MLD or MLQ during most of the passive aeration sequence (Fig. 2, Fig. S4). However, during forced aeration, the soluble concentration of these elements increased above the detection limits and was followed by a decrease of their particulate concentration. In particular, the soluble Mo concentration prevailed in its total content during forced aeration (Fig. S4). Finally, As displayed a slightly different behavior. Although its soluble concentration is nearly constant during the first 22 days of passive aeration, a marked increase was observed from day 29. This increase is followed by a decrease of its particulate concentration. Unlike other elements, forced aeration had no significant impact on As soluble concentration.

Overall, aeration induces a release in solution of all quantified elements (*i.e.* Al, As, Co, Cr, Cu, Fe, Mn, Mo and Pb). This release was likely caused by direct oxidation of sulfur precipitates in presence of oxygen from the air (Fermoso et al., 2015). However, oxidation performed by indigenous microorganisms such as sulfur oxidizing bacteria (*i.e. Acidithiobacillus* species) (Jain and Tyagi, 1992) is not excluded, though this hypothesis needs further investigations. In both cases, sulfide oxidation leads to metal sulfide precipitates dissolution (*e.g.* FeS, CoS, Cu_2S, PbS) (Maharaj et al., 2018; Möller and Müller, 2012) as well as the release of sulfate. Indeed, a significant increase of sulfate concentration was measured after the 57th days of passive aeration and during forced aeration (Fig. S5). These results are in agreement with the soluble sulfate in sludge suspension found by Carbonell-Barrachina et al. (1999) under oxidizing conditions. Regarding particulate As, it can be hypothesized that it is initially co-precipitated with Fe sulfides (Savage et al., 2000) and consequently released in solution after their dissolution upon oxidation. This is consistent with the slight increase of soluble Fe observed from the 29th day of passive aeration.

3.3. DGT-labile elements concentration

Labile concentrations of Cd, Cr(III), Cu and Pb were lower than 0.02, 0.2, 2, 0.6 µg/L, respectively, during the whole experiment. The labile concentrations of Mo, Sb and W were close or below the MLD_{DGT} during most of the passive aeration experiment (Fig. S5). Labile concentrations of the other elements are given in Fig. S5 and typical examples are displayed in Fig. 3. Labile Al, As, Co, Fe and Mn rapidly decreased during the first 3–5 days of passive aeration and later their concentration remained rather constant until the 57th day of aeration at least. Conversely, no initial decrease was observed for Ni and Se.

Under forced aeration, several elements (*i.e.* Al, Mo, Ni, Sb, Se and W) displayed a rapid increase of their DGT-labile concentrations followed by a decrease, except for Mo and W. As and Co slightly decreased immediately after forced aeration and their concentration increased again at the 85th day. After 57 days of passive aeration Fe and Mn behavior differs from the other elements since their labile concentrations continued to decrease, even under forced aeration.

The decrease of labile Al at the beginning of passive aeration may be explained by the presence of negatively charged hydroxide complexes (*e.g.* $Al(OH)_4^-$) at pH 7.8 ± 0.3 that are not efficiently sampled by

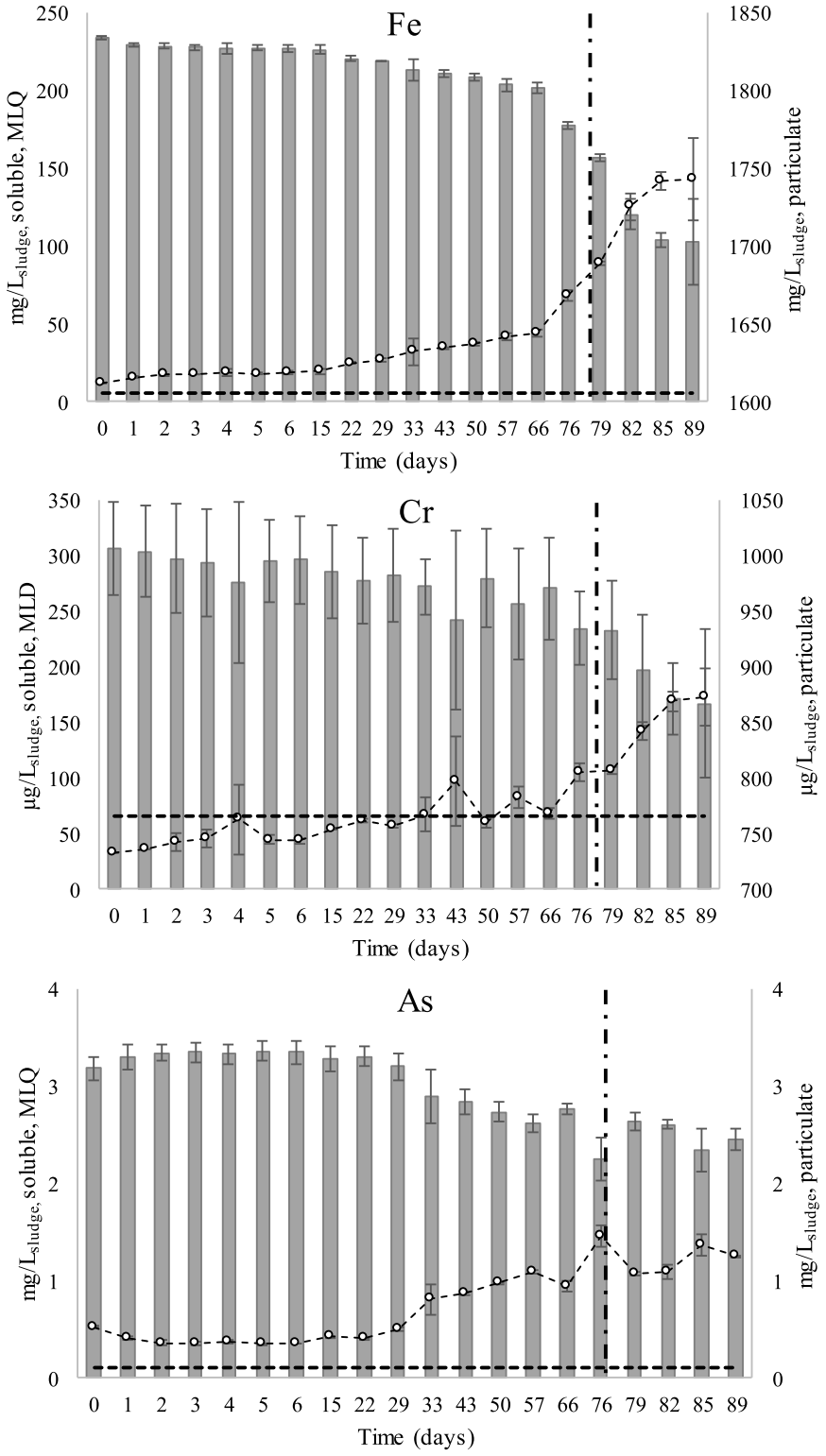


Fig. 2. Examples of soluble (dashed line with circles) and particulate (bars) elements' concentration over time. The bold horizontal dashed line is the method limit of detection (MLD) or quantification (MLQ) for soluble elements whereas the vertical dashed line indicates the beginning of forced aeration.

Chelex-DGT (Panther et al., 2012). The increase of labile Al, As, Co, Ni after 57 days of aeration could be a direct consequence of their release form sulfide species as discussed in 3.2. In contrast, the decrease of Fe and Mn labile concentration is not associated with the increase of their soluble fraction, especially at the end of the forced aeration, meaning that part of these soluble elements are DGT-inert (*e.g.* colloids such as Fe(II)-phosphate or strongly complexed with organic functional groups such as thiol groups (Shakeri Yekta et al., 2014)). Therefore, it can be concluded that oxidation converts a part of labile species of Fe and Mn into soluble non-labile species. Similarly, Øygard et al. (2007) showed a strong decrease of labile Fe (determined with cation exchange SPE cartridge) during the exposition of leachate to oxygen, while particulate and colloidal Fe (*e.g.* iron oxides) increased.

Conversely, the delay observed for the increase of labile As and Co

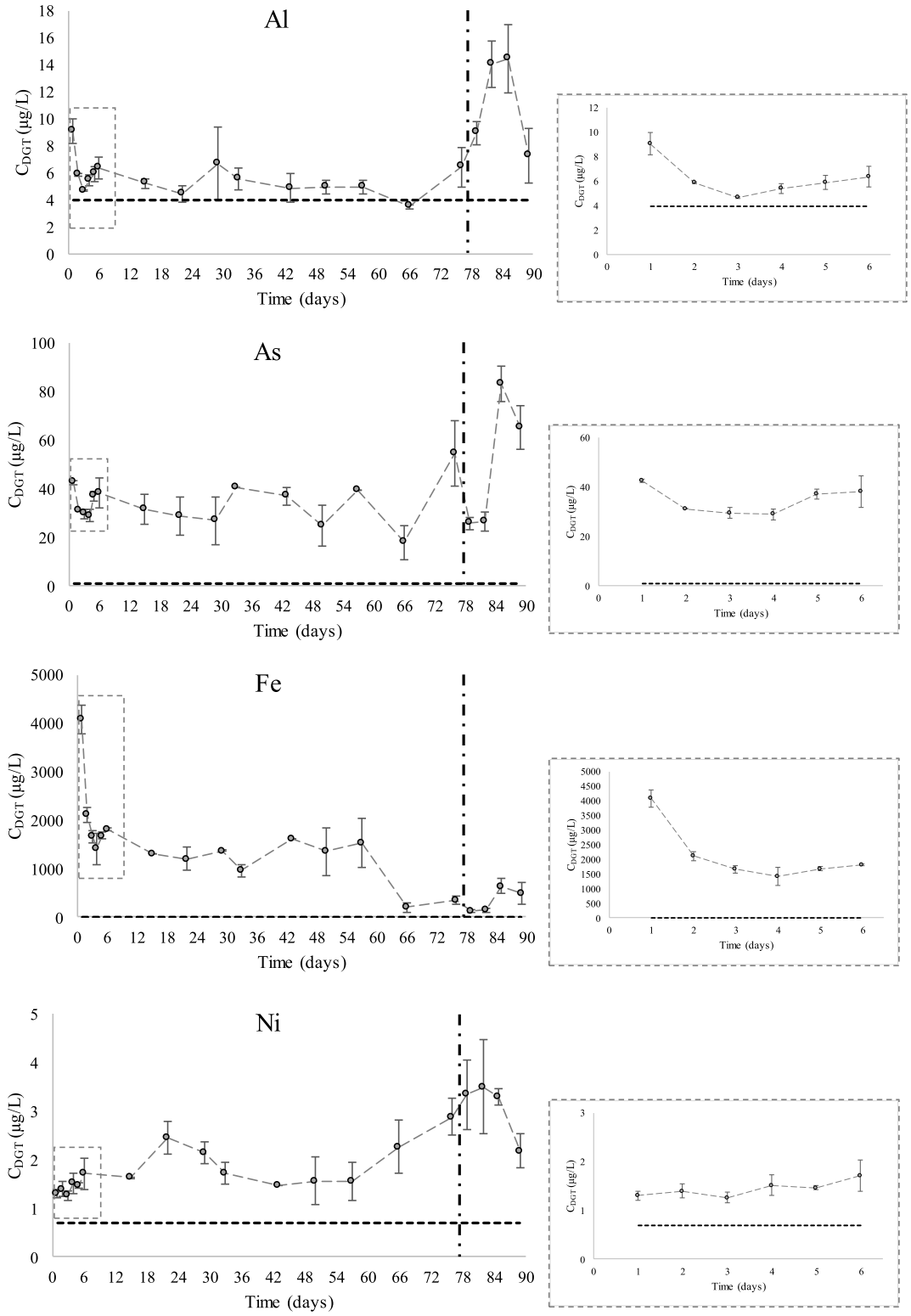


Fig. 3. Examples of labile elements' concentration over time. The bold horizontal dashed line is the MLQ_{DGT} whereas the vertical dashed line indicates the beginning of forced aeration. The inset is an enlargement of the first 6 days of the experiment.

concentration during forced aeration let suppose slow mechanisms of conversion into labile form. Moreover, adsorption onto Fe/Mn colloids could have occurred.

3.4. Environmental impact of digestate aeration

In this study, performed at laboratory-scale in controlled conditions, it was reported that aeration regime modifies TEs distribution among labile, soluble and particulate fractions. It is assumed that the observed TEs' fractionation can help to anticipate phenomena related to air exposure occurring on field during digestate management. Indeed, the passive aeration experiment could show the phenomena that can be expected during the storage of digestate in open tanks. Usually, the required storage time of digestate before land spreading may range

from 90 days to 10 months depending on the country and digestate spreading season (Plana and Noche, 2016). The variation on TEs' mobility observed during forced aeration is hypothesized to be similar to the one occurring during digestate handling for land application since the contact between air and digestate is significant.

Passive and forced aeration resulted both in a release in solution of Al, As, Co, Cr, Cu, Fe, Mn, Mo and Pb. Therefore, aeration of digestate could increase mobility of TEs over time. Under passive aeration, dissolution was slow during the first four weeks. Consequently, storage of digestate in an open tank could increase only marginally TEs mobility provided the storage duration is limited. However, dissolution increased significantly after approximately 30 days of passive aeration for most elements. Such increase is likely correlated to the increase of the surface to volume ratio (from 0.39 dm^{-1} to 0.45 dm^{-1} after 30 days of aeration) that controlled the rate of aeration of the digestate during the experiment. Therefore, design of digestate storage tank would be an important parameter to limit the increase of trace element mobility during storage. In this context, digestate storage tank with low surface to volume ratio (*i.e.* important height) should be favored. Forced aeration resulted in an important dissolution of all the quantified elements, except for As. Therefore, it is assumed that TEs' mobility could be strongly increased during digestate handling for land spreading. A "safety factor" which counts for TEs' oxidation during digestate handling should be considered for environmental risk assessment.

Alongside particulate/soluble fractions, DGT-labile elements were measured during this study. DGT-labile species (*i.e.* free + weak complexes) are the most reactive species and would represent the most readily bio-accessible fraction of TEs (Zhang and Davison, 2015). During passive aeration, although soluble elements' concentration increased, no correlated increase of DGT-labile concentrations was found for Al, As, Co, Fe, Mn, and Se. Only DGT-labile Ni showed a small delayed increase ($\geq$60 days, within a factor 2). Therefore, storage of digestate in an open tank could have no impact on the labile fraction of most of the studied TEs.

During forced aeration, except for Fe and Mn, all quantified labile elements rapidly increased. Moreover, the bio-accessibility of labile elements could increase after land application depending on the soils' sorption capacity (Dragicevic et al., 2018b; Kabata-Pendias, 2004) and plants uptake mechanisms (Lehto et al., 2006; Tack, 2010). Such hypothesis should be further studied for risk assessment. It was also observed that labile Al, As, Co, Ni, Sb and Se decreased after one week of forced aeration, therefore, it is not excluded that their bio-accessibility could remain unaltered during digestate land application.

4. Conclusions

In this work, the influence of aeration of sewage sludge digestate on the fractionation of fourteen TEs (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se and W) was studied with a laboratory-scale tank. Aeration promoted dissolution of all the quantified elements (*i.e.* Al, As, Co, Cr, Cu, Fe, Mn, Mo and Pb), which was certainly due to oxidation of metal sulfide precipitates. Therefore, it was assumed that the observed increase of TEs mobility due to aeration may likely occur during storage in open tank or digestate handling before land application. However, this dissolution did not promote an increase of DGT-labile concentrations during passive aeration. Conversely, forced aeration promoted an increase of the labile Al, As, Co, Mo, Ni, Sb, Se and W. Therefore, it can be assumed that passive aeration of digestate like in open storage tank would not increase TEs bio-accessibility unless significant aeration such as during digestate handling for land spreading takes place.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

Patrice Fondanèche is acknowledged for his assistance in the laboratory, especially during ICP-MS and MP-AES analyses.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2019.02.120.

Funding sources

This work was supported by the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 643071.

References

Arai, Y., 2010. Arsenic and antimony. In: Trace Elements in Soils. Wiley-Blackwell, pp. 381–407. https://doi.org/10.1002/9781444319477.ch16.

Boulamanti, A.K., Donida Maglio, S., Giuntoli, J., Agostini, A., 2013. Influence of different practices on biogas sustainability. Biomass Bioenergy 53, 149–161. https://doi.org/10.1016/j.biombioe.2013.02.020.

Cao, W., Wang, M., Liu, M., Zhang, Z., Sun, Z., Miao, Y., Sun, C., Hu, C., 2018. The chemical and dynamic distribution characteristics of iron, cobalt and nickel in three different anaerobic digestates: effect of pH and trace elements dosage. Bioresour. Technol. 269, 363–374. https://doi.org/10.1016/j.biortech.2018.08.094.

Carbonell-Barrachina, A.A., Jugsujinda, A., Burlo, F., Delaune, R.D., Patrick Jr., W.H., 1999. Arsenic chemistry in municipal sewage sludge as affected by redox potential and pH. Water Res. 34, 216–224.

Charter, R.A., Tabatabai, M.A., Schafer, J.W., 1995. Arsenic, molybdenum, selenium, and tungsten contents of distinct fertilizers and phosphate rocks1. Commun. Soil Sci. Plant Anal. 26, 3051–3062. https://doi.org/10.1080/00103629509369508.

Devillers, D., Buzier, R., Simon, S., Charriau, A., Guibaud, G., 2016. Simultaneous measurement of Cr(III) and Cr(VI) in freshwaters with a single diffusive gradients in thin films device. Talanta 154, 533–538. https://doi.org/10.1016/j.talanta.2016.04.009.

Dragicevic, I., Eich-Greatorex, S., Sogn, T.A., Horn, S.J., Krogstad, T., 2018a. Use of high metal-containing biogas digestates in cereal production – Mobility of chromium and aluminium. J. Environ. Manag. 217, 12–22. https://doi.org/10.1016/j.jenvman.2018.03.090.

Dragicevic, I., Sogn, T.A., Eich-Greatorex, S., 2018b. Recycling of biogas digestates in crop production—soil and plant trace metal content and variability. Front. Sustain. Food Syst. 2, 1–14. https://doi.org/10.3389/fsufs.2018.00045.

European Commission, 2016. Sewage Sludge [WWW Document]. URL. http://ec.europa.eu/environment/waste/sludge/index.htm, Accessed date: 10 February 2018.

Fermoso, F.G., van Hullebusch, E.D., Guibaud, G., Collins, G., Svensson, B.H., Carliell-Marquet, C., Vink, J.P.M., Esposito, G., Frunzo, L., 2015. Fate of trace metals in anaerobic digestion. In: Guebitz, G.M., Bauer, A., Bochmann, G., Gronauer, A., Weiss, S. (Eds.), Biogas Science and Technology. Springer International Publishing, Switzerland, pp. 171–195. https://doi.org/10.1007/978-3-319-21993-6_7.

Fu, M.H., Tabatabai, M.A., 1988. Tungsten content of soils, plants, and sewage sludges in Iowa. J. Environ. Qual. 17, 146–148. https://doi.org/10.2134/jeq1988.00472425001700010024x.

Hamnér, K., Kirchmann, H., 2015. Trace element concentrations in cereal grain of long-term field trials with organic fertilizer in Sweden. Nutrient Cycl. Agroecosyst. 103, 347–358. https://doi.org/10.1007/s10705-015-9749-7.

Healy, M.G., Fenton, O., Forrestal, P.J., Danaher, M., Brennan, R.B., Morrison, L., 2016. Metal concentrations in lime stabilised, thermally dried and anaerobically digested sewage sludges. Waste Manag. 48, 404–408. https://doi.org/10.1016/j.wasman.2015.11.028.

Hooda, P.S., 2010. Assessing bioavailability of soil trace elements. In: Trace Elements in Soils, pp. 227–265. https://doi.org/10.1002/9781444319477.ch11.

Jain, D.K., Tyagi, R.D., 1992. Leaching of heavy metals from anaerobic sewage sludge by sulfur-oxidizing bacteria. Enzym. Microb. Technol. 14, 376–383. https://doi.org/10.1016/0141-0229(92)90006-A.

Kabata-Pendias, A., 2004. Soil-plant transfer of trace elements - An environmental issue. Geoderma 122, 143–149. https://doi.org/10.1016/j.geoderma.2004.01.004.

Kupper, T., Bürge, D., Bachmann, H.J., Güsewell, S., Mayer, J., 2014. Heavy metals in source-separated compost and digestates. Waste Manag. 34, 867–874. https://doi.org/10.1016/j.wasman.2014.02.007.

Laera, A., Buzier, R., Guibaud, G., Esposito, G., van Hullebusch, E.D., 2019. Assessment of the DGT technique in digestate to fraction twelve trace elements. Talanta 192, 204–211. https://doi.org/10.1016/j.talanta.2018.09.033.

Lehto, N.J., Davison, W., Zhang, H., Tych, W., 2006. Analysis of micro-nutrient behaviour in the rhizosphere using a DGT parameterised dynamic plant uptake model. Plant Soil 282, 227–238. https://doi.org/10.1007/s11104-005-5848-6.

Liebetrau, J., Clemens, J., Cuhls, C., Hafermann, C., Friehe, J., Weiland, P., Daniel-Gromke, J., 2010. Methane emissions from biogas-producing facilities within the agricultural sector. Eng. Life Sci. 10, 595–599. https://doi.org/10.1002/elsc.201000070.

Maharaj, B.C., Mattei, M.R., Frunzo, L., van Hullebusch, E.D., Esposito, G., 2018. ADM1 based mathematical model of trace element precipitation/dissolution in anaerobic digestion processes. Bioresour. Technol. 267, 666–676. https://doi.org/10.1016/j.biortech.2018.06.099.

McBride, M.B., 2003. Toxic metals in sewage sludge-amended soils: has promotion of beneficial use discounted the risks? Adv. Environ. Res. 8, 5–19. https://doi.org/10.1016/S1093-0191(02)00141-7.

Möller, K., Müller, T., 2012. Effects of anaerobic digestion on digestate nutrient availability and crop growth: a review. Eng. Life Sci. 12, 242–257. https://doi.org/10.1002/elsc.201100085.

Ortner, M., Rachbauer, L., Somitsch, W., Fuchs, W., 2014. Can bioavailability of trace nutrients be measured in anaerobic digestion? Appl. Energy 126, 190–198. https://doi.org/10.1016/j.apenergy.2014.03.070.

Øygard, J.K., Gjengedal, E., Røyset, O., 2007. Size charge fractionation of metals in municipal solid waste landfill leachate. Water Res. 41, 47–54. https://doi.org/10.1016/j.watres.2006.09.027.

Panther, J.G., Bennett, W.W., Teasdale, P.R., Welsh, D.T., Zhao, H., 2012. DGT measurement of dissolved aluminum species in waters: comparing chelex-100 and titanium dioxide-based adsorbents. Environ. Sci. Technol. 46, 2267–2275. https://doi.org/10.1021/es203674n.

Plana, P.V., Noche, B., 2016. A review of the current digestate distribution models: storage and transport. In: 8Th International Conference on Waste Management and The Environment, pp. 345–357. https://doi.org/10.2495/WM160311.

Riva, C., Orzi, V., Carozzi, M., Acutis, M., Boccasile, G., Lonati, S., Tambone, F., D'Imporzano, G., Adani, F., 2016. Short-term experiments in using digestate products as substitutes for mineral (N) fertilizer: Agronomic performance, odours, and ammonia emission impacts. Sci. Total Environ. 547, 206–214. https://doi.org/10.1016/j.scitotenv.2015.12.156.

Savage, K.S., Tingle, T.N., O'Day, P.A., Waychunas, G.A., Bird, D.K., 2000. Arsenic speciation in pyrite and secondary weathering phases, Mother lode gold district, Tuolumne County, California. Appl. Geochem. 15, 1219–1244. https://doi.org/10.1016/S0883-2927(99)00115-8.

Scarlat, N., Dallemand, J.F., Fahl, F., 2018. Biogas: developments and perspectives in Europe. Renew. Energy 129, 457–472. https://doi.org/10.1016/j.renene.2018.03.006.

Shakeri Yekta, S., Svensson, B.H., Björn, A., Skyllberg, U., Yekta, S.S., Svensson, B.H., Björn, A., Skyllberg, U., 2014. Thermodynamic modeling of iron and trace metal solubility and speciation under sulfidic and ferruginous conditions in full scale continuous stirred tank biogas reactors. Appl. Geochem. 47, 61–73. https://doi.org/10.1016/j.apgeochem.2014.05.001.

Tack, F.M.G., 2010. Trace elements: general soil chemistry, principles and processes. In: Hooda, P. (Ed.), Trace Elements in Soils. Wiley-Blackwell, pp. 9–37. https://doi.org/10.1002/9781444319477.ch2.

Tampio, E., Salo, T., Rintala, J., 2016. Agronomic characteristics of five different urban waste digestates. J. Environ. Manag. 169, 293–302. https://doi.org/10.1016/j.jenvman.2016.01.001.

van Hullebusch, E.D., Guibaud, G., Simon, S., Lenz, M., Yekta, S.S., Fermoso, F.G., Jain, R., Duester, L., Roussel, J., Guillon, E., Skyllberg, U., Almeida, C.M.R., Pechaud, Y., Garuti, M., Frunzo, L., Esposito, G., Carliell-Marquet, C., Ortner, M., Collins, G., 2016. Methodological approaches for fractionation and speciation to estimate trace element bioavailability in engineered anaerobic digestion ecosystems: an overview. Crit. Rev. Environ. Sci. Technol. 46, 1324–1366. https://doi.org/10.1080/10643389.2016.1235943.

Vrana, B., Allan, I.J., Greenwood, R., Mills, G.A., Dominiak, E., Svensson, K., Knutsson, J., Morrison, G., 2005. Passive sampling techniques for monitoring pollutants in water. TrAC Trends Anal. Chem. (Reference Ed.). https://doi.org/10.1016/j.trac.2005.06.006.

Zhang, H., Davison, W., 2015. Use of diffusive gradients in thin-films for studies of chemical speciation and bioavailability. Environ. Chem. 12, 85–101. https://doi.org/10.1071/EN14105.

Zhang, H., Davison, W., 1999. Diffusional characteristics of hydrogels used in DGT and DET techniques. Anal. Chim. Acta 398, 329–340. https://doi.org/10.1016/S0003-2670(99)00458-4.

Zhang, H., Davison, W., 1995. Performance characteristics of diffusion gradients in thin films for the *in situ* measurement of trace metals in aqueous solution. Anal. Chem. 67, 3391–3400. https://doi.org/10.1021/ac00115a005.

Zhang, H., Davison, W., Knight, B., Mcgrath, S., 1998. In situ measurements of solution concentrations and fluxes of trace metals in sells using DGT. Environ. Sci. Technol. 32, 704–710. https://doi.org/10.1021/es9704388.

Supporting information for

Distribution trend of trace elements in digestate exposed to air: laboratory-scale investigations using DGT-based fractionation

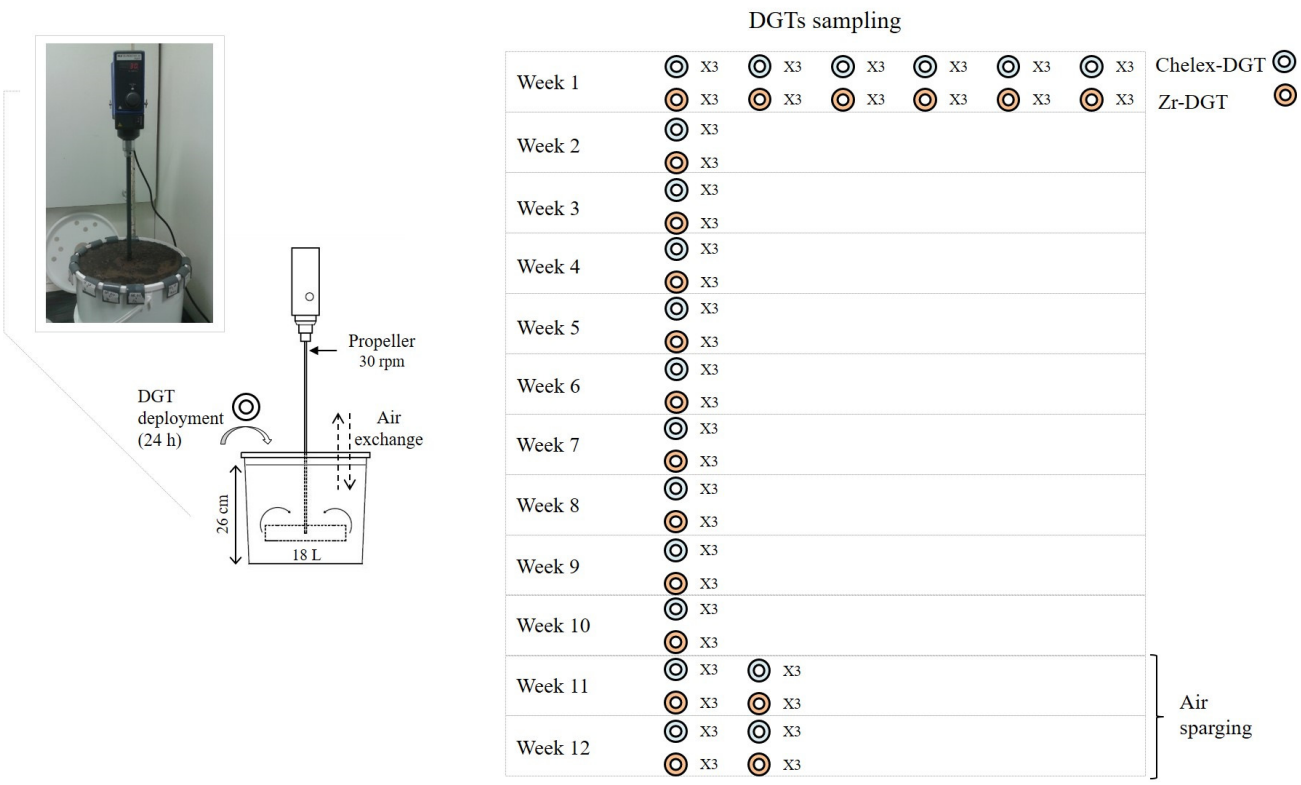


Figure S1. On the left, the pilot scale tank containing the digested sludge. On the right, a scheme of Chelex and Zr-DGTs sampling throughout the experiment.

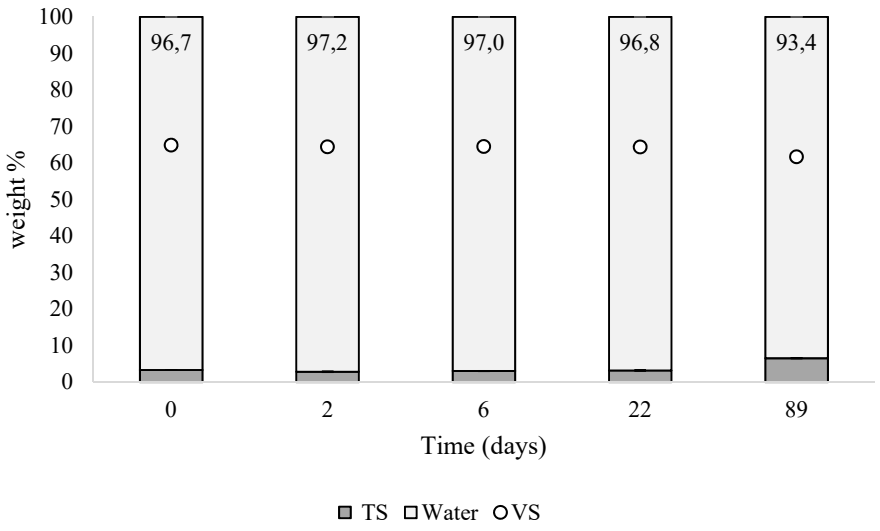


Figure S2. TS, VS and water concentrations (%) in digested sewage sludge at different sampling time.

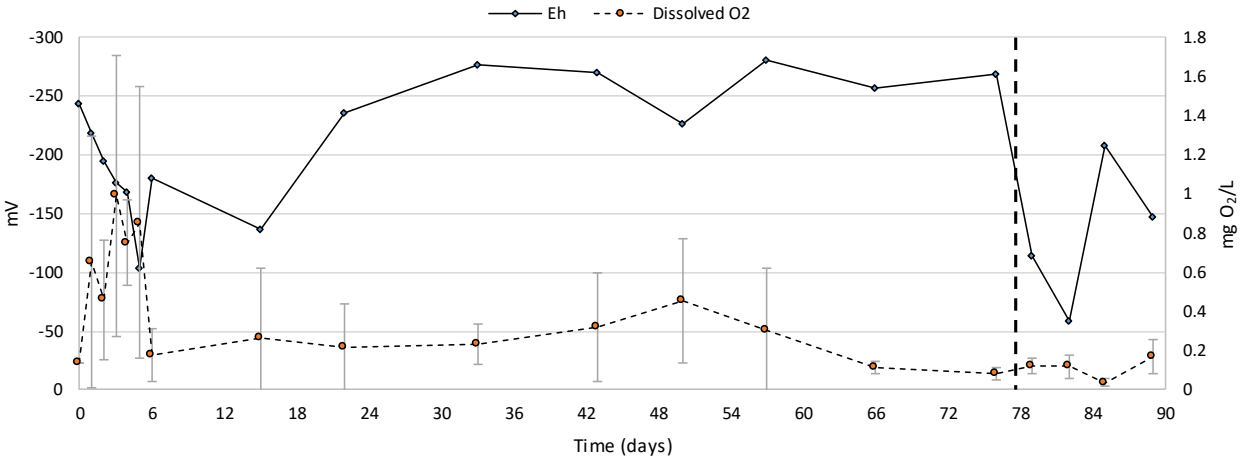
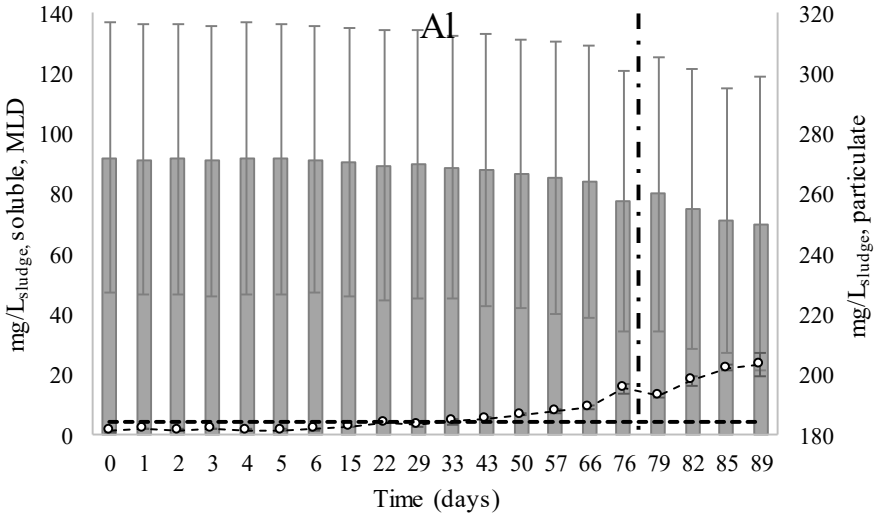
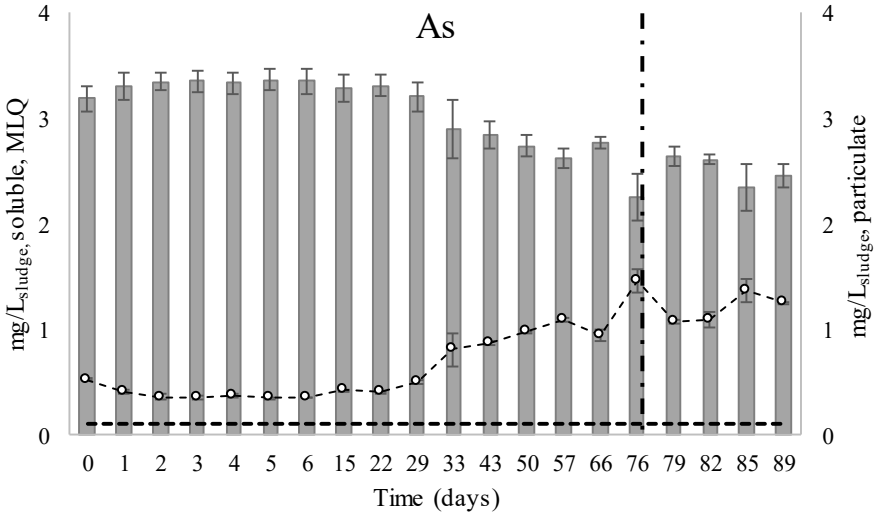
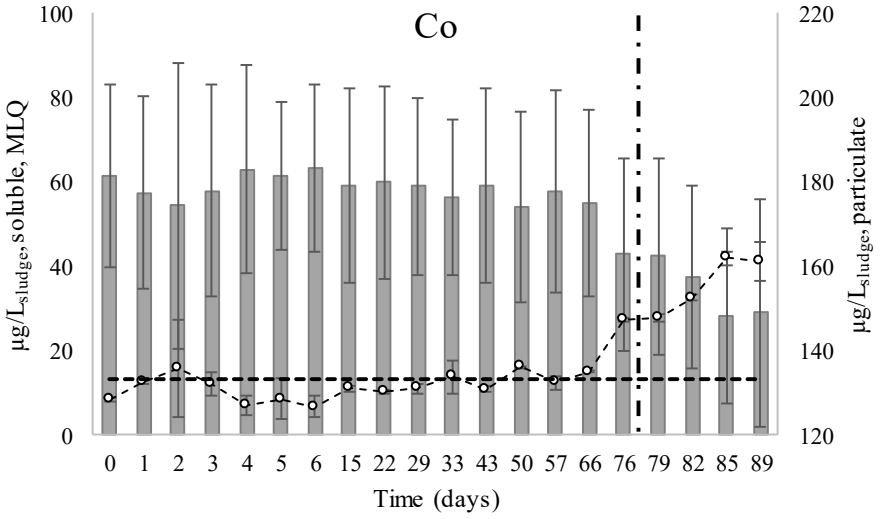
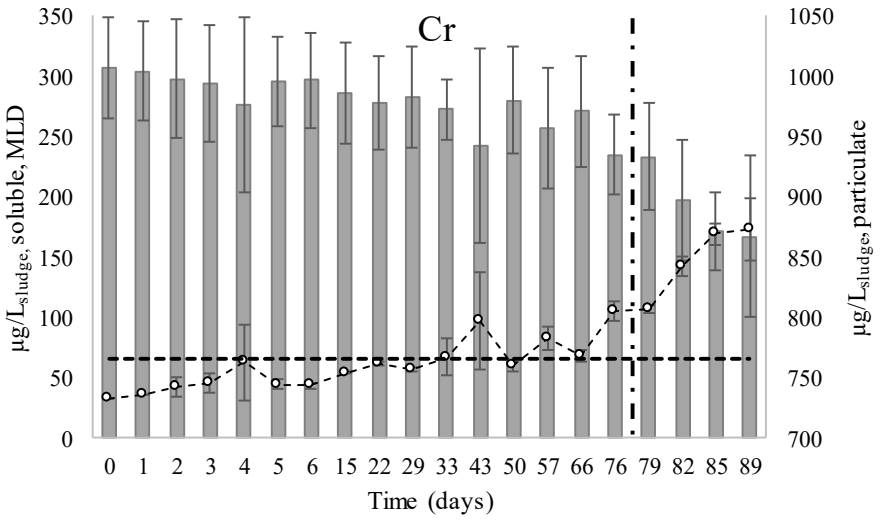


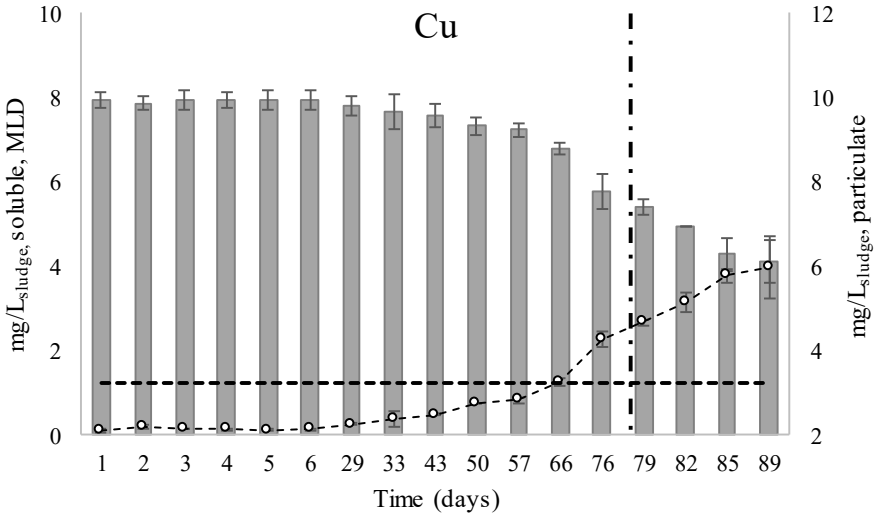
Figure S3. E_h and dissolved O_2 measured after retrieval of DGTs from the digested sludge. Dissolved O_2 is an average of triplicate measurements. The vertical dashed line indicates the beginning of forced aeration.

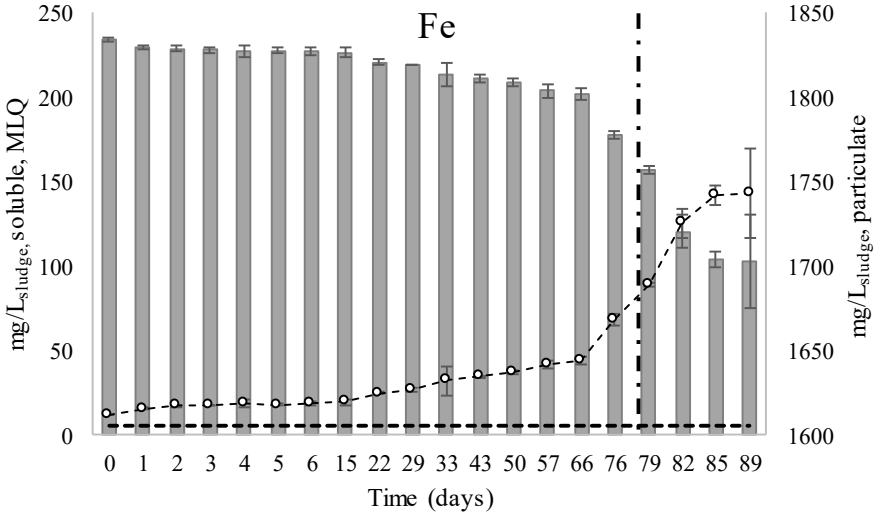

Al
mg/L$_{sludge}$, soluble, MLD
mg/L$_{sludge}$, particulate
0 20 40 60 80 100 120 140
180 200 220 240 260 280 300 320
0 1 2 3 4 5 6 15 22 29 33 43 50 57 66 76 79 82 85 89
Time (days)

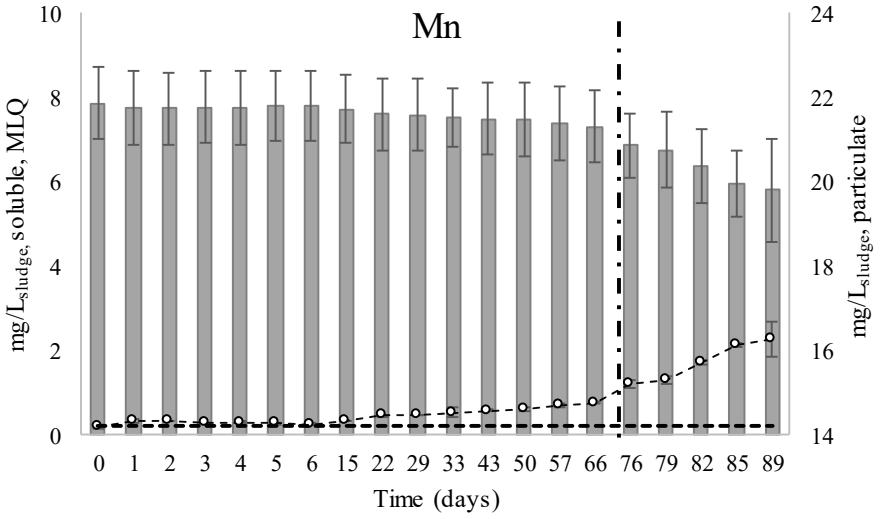

As
mg/L$_{sludge}$, soluble, MLQ
mg/L$_{sludge}$, particulate
0 1 2 3 4
0 1 2 3 4
0 1 2 3 4 5 6 15 22 29 33 43 50 57 66 76 79 82 85 89
Time (days)

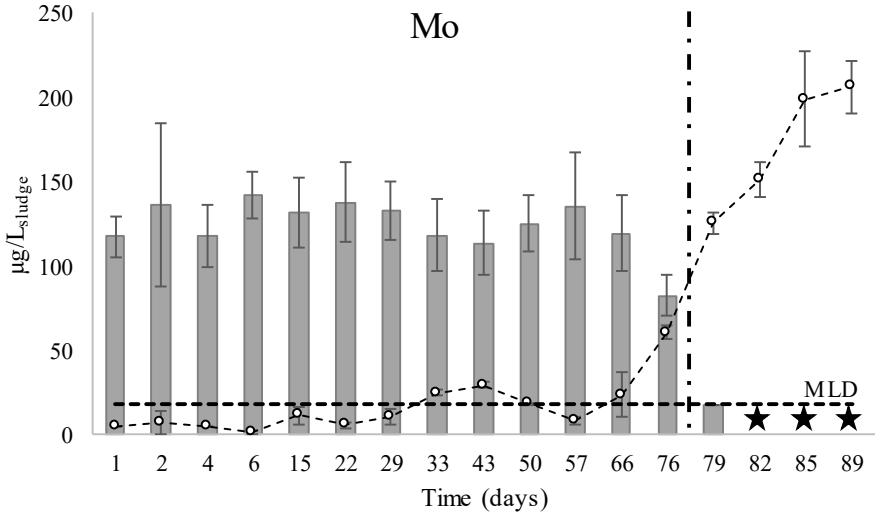

Co
µg/L$_{sludge}$, soluble, MLQ
µg/L$_{sludge}$, particulate
Time (days)
0 1 2 3 4 5 6 15 22 29 33 43 50 57 66 76 79 82 85 89
0 20 40 60 80 100
120 140 160 180 200 220


Cr
µg/L$_{sludge}$, soluble, MLD
µg/L$_{sludge}$, particulate
Time (days)
0 1 2 3 4 5 6 15 22 29 33 43 50 57 66 76 79 82 85 89
0 50 100 150 200 250 300 350
700 750 800 850 900 950 1000 1050


Cu
mg/L$_{sludge}$, soluble, MLD
mg/L$_{sludge}$, particulate
0
2
4
6
8
10
12
1 2 3 4 5 6 29 33 43 50 57 66 76 79 82 85 89
Time (days)


Fe
mg/L$_{sludge}$, soluble, MLQ
mg/L$_{sludge}$, particulate
0
50
100
150
200
250
1600
1650
1700
1750
1800
1850
0 1 2 3 4 5 6 15 22 29 33 43 50 57 66 76 79 82 85 89
Time (days)


Mn
mg/L$_{sludge}$, soluble, MLQ
mg/L$_{sludge}$, particulate
10
8
6
4
2
0
24
22
20
18
16
14
0 1 2 3 4 5 6 15 22 29 33 43 50 57 66 76 79 82 85 89
Time (days)


Mo
µg/L$_{sludge}$
250
200
150
100
50
0
MLD
1 2 4 6 15 22 29 33 43 50 57 66 76 79 82 85 89
Time (days)

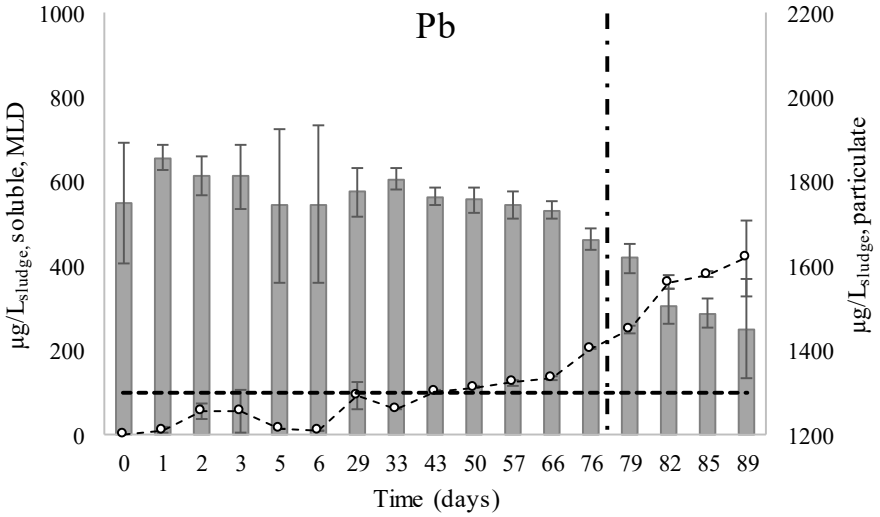


Figure S4. Soluble (dashed line with circles) and particulate (bars) elements' concentration over time. The bold horizontal dashed line is the method limit of detection (MLD) or quantification (MLQ) for soluble elements whereas the vertical dashed line indicates the beginning of forced aeration. When the soluble prevails the particulate fraction a black star replaces the bar.

Sulfate analysis

The supernatant recovered during the TSS and VSS analysis was filtered through 0.2 µm polyethersulfone (PES) syringe filters (Pall Laboratory) to further measure sulfate. Sulfate was measured by turbidimetric method using the sulfate test kit (SulfaVer 4 Method, HACH) and a spectrophotometer (DR 1900, HACH LANGE) at 450 nm.

To check the accuracy of the method, we prepared a 50 mg/L standard SO_4^{2-} solution from $Na_2SO_{4(s)}$ (Prolabo, Normapure). The recovery for SO_4^{2-} standard was equal or above 92%.

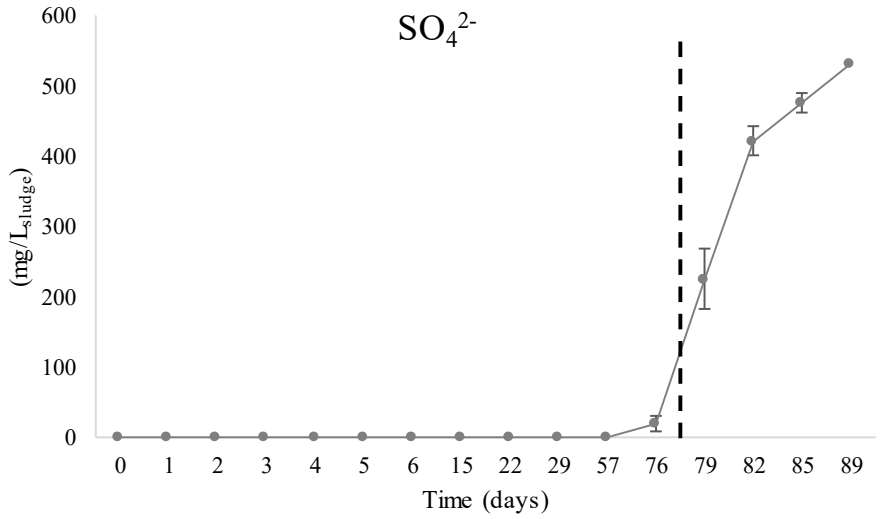
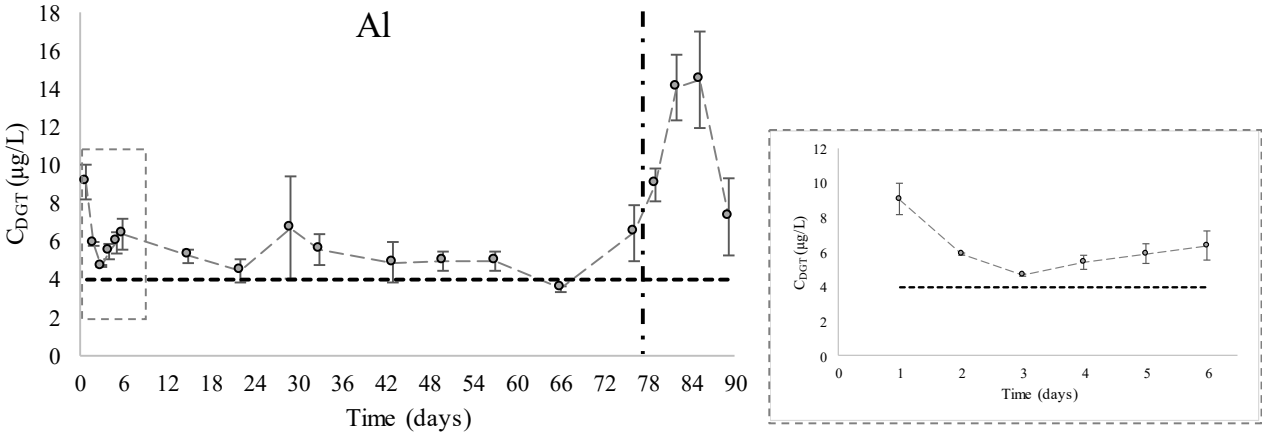
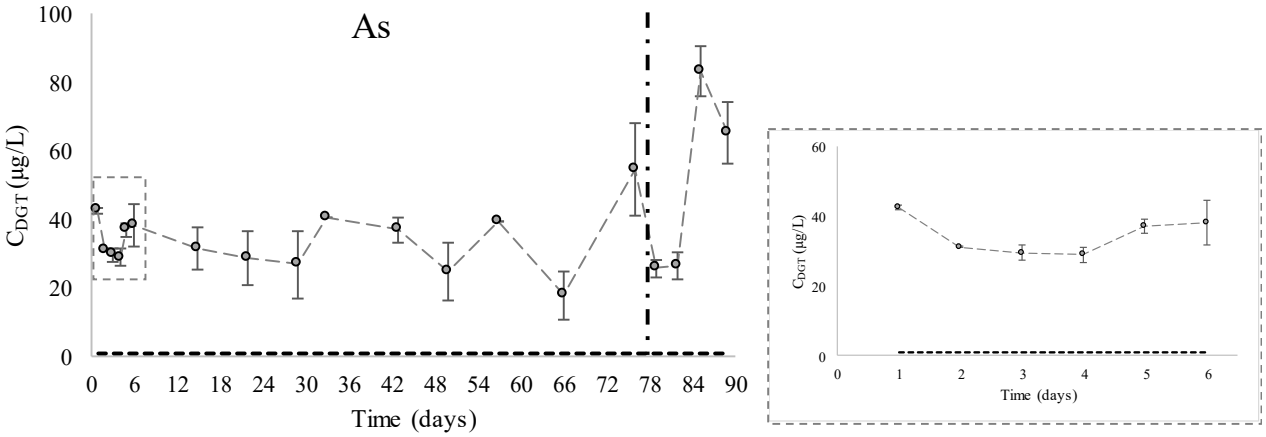
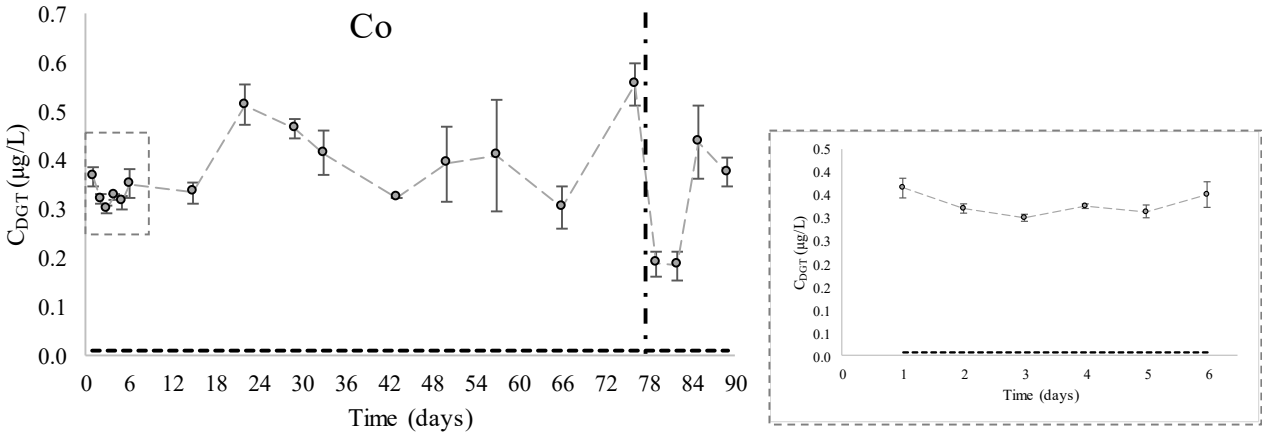
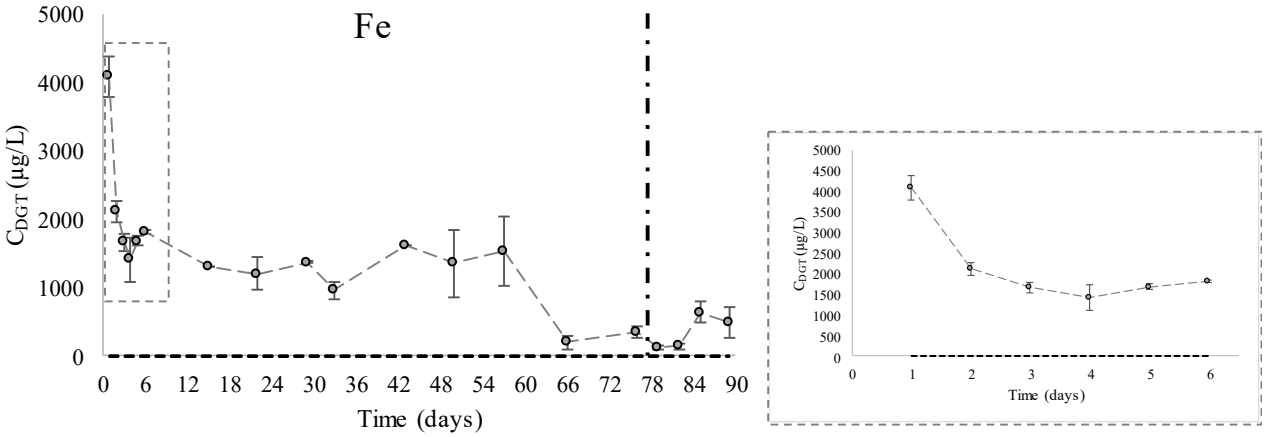


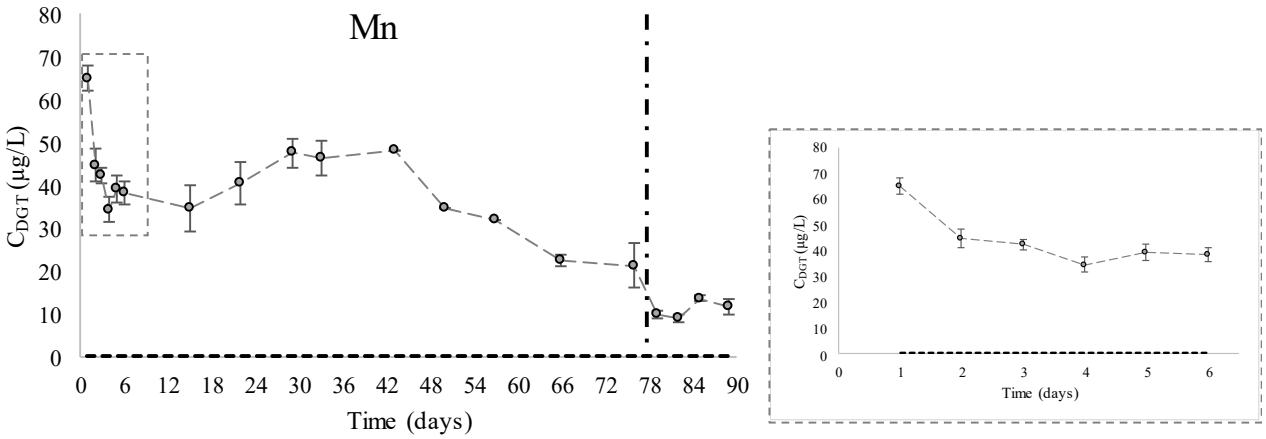
Figure S5. Sulfate measured over time. The vertical dashed line indicates the beginning of forced aeration.

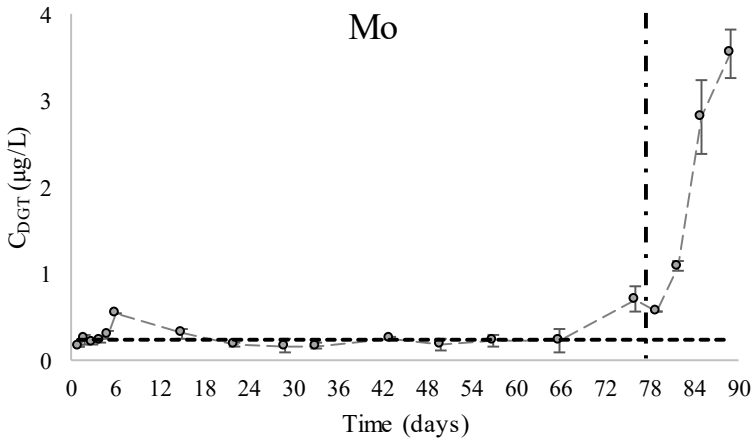

Al
C_{DGT} (µg/L)
Time (days)

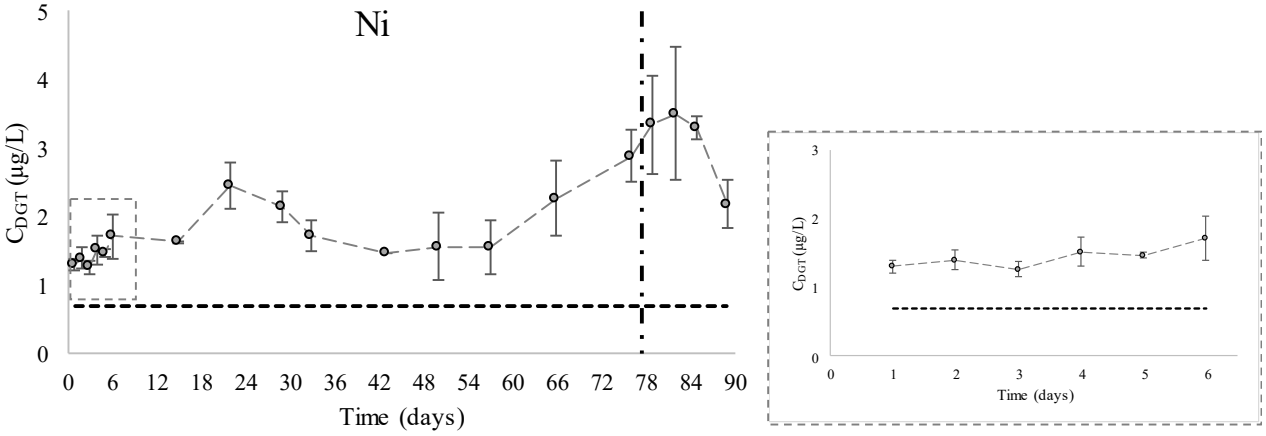

As
C_{DGT} (µg/L)
Time (days)

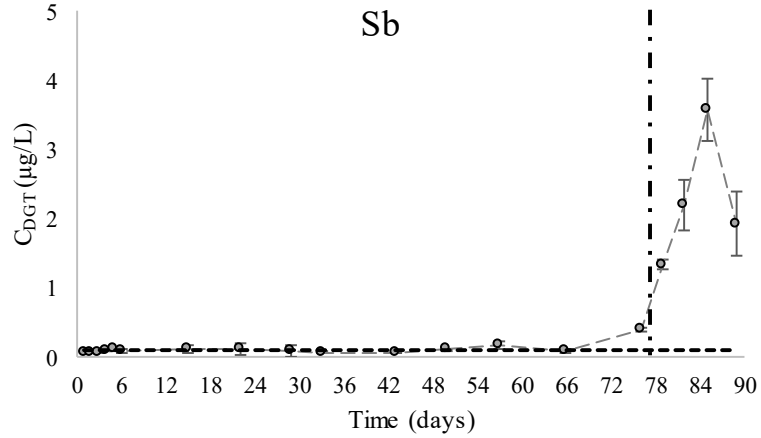

Co
C_{DGT} (µg/L)
Time (days)

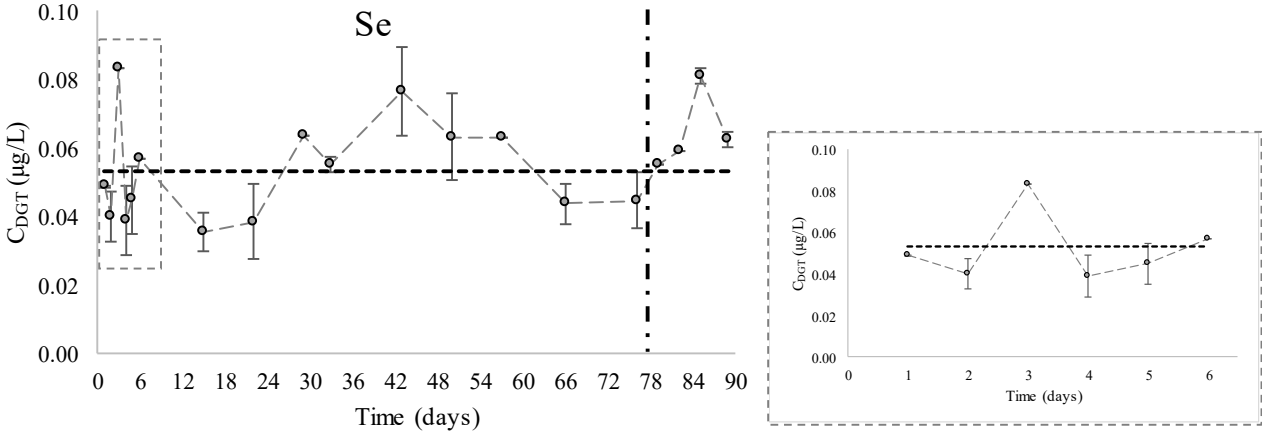

Fe
C_{DGT} (µg/L)
Time (days)


Mn
C_{DGT} (µg/L)
Time (days)


Mo
C_{DGT} (µg/L)
Time (days)


Ni
C_{DGT} (µg/L)
Time (days)


Sb
C_{DGT} (µg/L)
Time (days)


Se
C_{DGT} (µg/L)
Time (days)

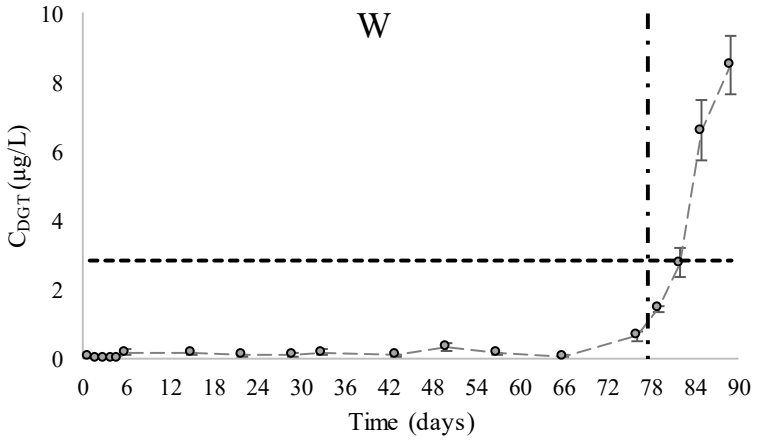


Figure S6. Labile trace elements (grey dashed line with grey circles) monitored over time in digested sewage sludge. The bold horizontal dashed line is the MLD_{DGT} or MLQ_{DGT} whereas the vertical dashed line indicates the beginning of forced aeration. The inset is an enlargement of the first 6 days of the experiment.

Table S1. Elution factors f_e from the literature with elution conditions similar to this work.

	Elution factor	Reference
Al	0.85	(Devillers et al., 2017)
As	0.70	Result not published
Cd	0.85	(Devillers et al., 2017)
Co	0.85	(Devillers et al., 2017)
Cr	0.80	(Devillers et al., 2017)
Cu	0.85	(Devillers et al., 2017)
Fe	0.70	(Zhang and Davison, 1995)
Mn	0.82	(Zhang and Davison, 1995)
Mo	0.86	Result not published
Ni	0.85	(Devillers et al., 2017)
Pb	0.85	(Devillers et al., 2017)
Sb	0.61	Result not published
Se	0.86	Result not published
W	0.70	Result not published

Table S2. Coefficients of diffusion in a standard diffusive gel taken from the literature and used in this study. All values are referred to 25°C.

	$D_{standard}$ (cm^2/sec)
Al	$4.75 \cdot 10^{-6a}$
As	$6.90 \cdot 10^{-6b}$
Cd	$6.09 \cdot 10^{-6a}$
Co	$5.94 \cdot 10^{-6a}$
Cr (III)	$5.05 \cdot 10^{-6a}$
Cu	$6.23 \cdot 10^{-6a}$
Fe	$6.11 \cdot 10^{-6a}$
Mn	$5.85 \cdot 10^{-6a}$
Mo	$6.62 \cdot 10^{-6b}$
Ni	$5.77 \cdot 10^{-6a}$
Pb	$8.03 \cdot 10^{-6a}$
Sb	$6.92 \cdot 10^{-6b}$
Se	$7.77 \cdot 10^{-6b}$
W	$6.05 \cdot 10^{-6b}$

[a] Reference: http://www.dgtresearch.com/diffusion-coefficients/

Limits of detection/quantification

The limits of detection/quantification following acid digestion and DGT sampling were determined to account for sample contamination. The method's limits of detection and quantification (namely MLD and MLQ for total and soluble elements, Table S1, MLD_{DGT} and MLQ_{DGT} for labile elements, Table S2) were calculated according to IUPAC as the average plus three or ten times the standard deviation of the blanks, respectively. For the acid digestion procedure (total and soluble elements) we used 18 blanks, whereas for DGT sampling (labile elements) we used 25 blanks.

Table S3. The method's limits of detection (MLD) and quantification (MLQ) for total and soluble elements. MLD and MLQ are expressed on the same concentration basis as those for the samples using 0.29 $L/g_{TSinitial}$ and 0.25 L/L_{sludge} as conversion factor for total and soluble elements, respectively.

	$MLD_{soluble}$ ($\mu g/L_{sludge}$)	MLD_{total} ($\mu g/g_{TSin}$)	$MLQ_{soluble}$ ($\mu g/L_{sludge}$)	MLQ_{total} ($\mu g/g_{TSin}$)
Al	4420	51	10618	123
As	43	0.5	99	1.1
Cd	5	0.1	12	0.2
Co	5.4	0.1	13	0.2
Cr	66	1	117	1.4
Cu	1233	14	3257	38
Fe	1946	23	4806	56
Mn	91	1	201	2
Mo	18	0.2	47	0.5
Ni	721	8	1709	20
Pb	101	1	237	3
Sb	102	1	269	3
Se	1077	12	2630	30
W	69	1	181	2

Table S4. DGT method limit of detection (MLD_{DGT}) and quantification (MLQ_{DGT}) for a 24h deployment at 19°C (average of recorded values during all deployments). The values are calculated using Eq. (1).

	MLD_{DGT} (µg/L)	MLQ_{DGT} (µg/L)
Al	2	4
As	0.3	0.7
Cd	0.02	0.04
Co	0.003	0.01
Cr	0.1	0.2
Cu	1	2
Fe	1	2
Mn	0.2	0.4
Mo	0.2	0.5
Ni	0.3	0.7
Pb	0.2	0.6
Sb	0.1	0.3
Se	0.02	0.1
W	3	7

Table S5. Total element content of the digested sludge at the beginning and the end of the experiment. Results are mean of duplicates ± standard deviation.

	Beginning (µg/g_{TSin})	End (µg/g_{TSin})
Al	9070 ± 1420	10119 ± 1489
As	123 ± 5	123 ± 5
Cd	1 ± 1	2 ± 1
Co	6 ± 1	6 ± 1
Cr	35 ± 1	36 ± 1
Cu	334 ± 10	357 ± 19
Fe	61343 ± 405	56513 ± 6677
Mn	733 ± 22	866 ± 67
Mo	5 ± 1	7 ± 1
Ni	<19*	26 ± 1
Pb	62 ± 1	64 ± 7
Sb	<3*	4 ± 1
Se	<12[#]	<12[#]
W	3 ± 1	3 ± 1

*MLQ=average blanks ± 10*standard deviation blanks (n=18)

References

Devillers, D., Buzier, R., Charriau, A., Guibaud, G., 2017. Improving elution strategies for Chelex®-DGT passive samplers. Anal. Bioanal. Chem. 409, 7183–7189. https://doi.org/10.1007/s00216-017-0680-4

Wang, Y., Ding, S., Xu, D., Wang, Y., Li, Y., Gong, M., Zhang, C., 2016. Simultaneous measurements of eight oxyanions using high-capacity diffusive gradients in thin films (Zr-Oxide DGT) with a high-efficiency elution procedure. Environ. Sci. Technol. 50, 7572–7580. https://doi.org/10.1021/acs.est.6b00206

Zhang, H., Davison, W., 1995. Performance Characteristics of Diffusion Gradients in Thin Films for the in Situ Measurement of Trace Metals in Aqueous Solution. Anal. Chem. 67, 3391–3400. https://doi.org/10.1021/ac00115a005